FINAL REPORT:MIPR E5293N159

PROPAGATION AND BIOLOGY OF CRYPTOBIOTIC CRUSTS - 93

December 31, 1993

E. Durant McArthur
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To: US Army Corps of Engineers
Construction Engineering Research Laboratory
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EFFECTS OF COMPRESSIONAL SURFACE DISTURBANCES ON NITROGENASE ACTIVITY IN CRYPTOBIOTIC SOIL CRUSTS

EFFECTS OF COMPRESSIONAL SURFACE DISTURBANCES ON NITROGENASE ACTIVITY IN CRYPTOBIOTIC CRUSTS

AND

MICROBIAL ANALYSIS OF REVEGETATION PLOTS IN CANYONLANDS NATIONAL PARK

FINAL REPORT INT-92-686-IA

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EFFECTS OF COMPRESSIONAL SURFACE DISTURBANCES
PART I: DUGWAY PROVING GROUNDS, UTAH

Surface Disturbance of Cryptobiotic Soil Crusts: Nitrogenase Activity, Chlorophyll Content, and Chlorophyll Degradation

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Running title: Surface disturbance and cryptobiotic crusts

Abstract

Cryptobiotic soil crusts are an important component of semi-arid and arid ecosystems. An important role of these crusts is the contribution of fixed nitrogen to cold-desert ecosystems. This study examines the residual effects of various intensities and combinations of different surface disturbances (raking, scalping, and tracked vehicles) on nitrogenase activity, chlorophyll content and chlorophyll degradation in these soil crusts. Nine months after disturbance chlorophyll content of disturbed soils was not statistically different from undisturbed controls, except in the scalped treatments, indicating recovery of this characteristic is fairly quick unless surface material is removed. Differences in chlorophyll degradation among treatments were not statistically significant. However, nitrogenase activity in all treatments showed tremendous reductions, ranging from 77-97%, when compared to the control, indicating this characteristic is slow to recover. Consequently, assessment of crustal recovery from disturbance must include not only visual and biomass characteristics, but other physiological measurements as well. Areas dominated by these crusts should managed conservatively until the implications of crustal disturbance is better understood.

Key words: cryptobiotic soil crusts, nitrogen cycles, cryptogamic crusts, microphytic crusts, disturbance, arid lands

Introduction

Cyanobacterial-lichen soil crusts are common in semi-arid and arid landscapes of the world, representing over 70% of the living cover in some of these systems. These crusts contribute in many ways to the ecosystems in which they occur, including enhancement of soil surface resistance to water and wind erosion, increased aggregation of soil particles and increased seedling establishment and survival of some species (Harper and Marble 1988; Johansen 1993; Belnap and Gardner 1993).

In addition, cryptobiotic crusts can also improve the nutrient status of soils. Many cyanobacteria and cyanobacterial components of soil lichens fix atmospheric nitrogen (Belnap 1992; Skujins and Klubek 1978; Terry and Burns 1987; West and Skujins 1977). Studies utilizing stable isotopes of nitrogen have demonstrated that nitrogen fixed by cyanobacteria in crusts is available to, and used by, neighboring vascular plants (Mayland et al. 1966; Mayland and McIntosh 1966). Plants grown in such crusts show higher concentrations of many essential macronutrients than plants of the same species grown in uncrusted soils (Harper and Pendleton, 1993; Harper and Belnap, unpublished data).

Soil surface disturbances negatively affects the integrity and coverage of cyanobacterial crusts, since the filaments are brittle when dry and both the cyanobacteria and lichens are easily crushed (Harper and Marble 1988; Belnap, 1993a; Anderson et al., 1982b; Jeffries and Klopatek 1987; Callison et al., 1985; Cole 1991). Recovery rates have been found to depend on the type and extent of disturbance, the availability of nearby inoculation material, and on temperature and moisture regimes that follow disturbance events. Based on biomass and/or visual criteria, estimates for time to unaided recovery of cryptobiotic crusts from disturbance have varied widely, ranging from a few years up to 100 years. Belnap (1993a) estimated that crusts on sandy soils on the Colorado Plateau require at least 40 years for full recovery under stable soil conditions.

Up until now, the effects of disturbance on physiological processes in cryptobiotic crusts has not been examined, and the rate of recovery of these processes in natural ecosystems has been neglected. This study examines the effects of different kinds of disturbance on nitrogenase activity, chlorophyll

degradation and chlorophyll content of cyanobacterial rich soil crusts, and the rate of recovery of each parameter from the different kinds of disturbance.

Materials and Methods

Plots were located at Dugway Proving Grounds, 70 miles southwest of Salt Lake City, Utah (40° longitude, 113° latitude). Site characteristics include an elevation of 1400 m (4600'); annual precipitation is 19.4 cm (7.6"), with 52% falling between April and September; and annual mean temperatures are 10.8° C, with the warmest month (July) averaging 25.7° C. Soil at the study area are lacustrine fine silty loams of the Skumpah Series, a mesic mixed natrargids with a pH of 8.5-9.0. Quantification of plant and ground cover was done in July, 1991, before treatments were applied. These showed that the vegetation was dominated by Kochia americana(Wats.) and Atriplex confertifolia ((Torr. and Frem.) Wats.)), with vascular plant cover averaging 5.4% (S.E. 0.3%). Soils were covered by a well-developed cyanobacterial-lichen-moss soil crust, with cover for lichens averaging 24% (S.E. 0.5%) and mosses 7% (S.E. 0.3%). Of the lichens present, only Collema sp. is known to fix nitrogen; it represented approximately 50% of the lichen cover. Of the cyanobacteria present, Microcoleus vaginatus(Vauch.) Gom. was dominant, being over 95% of the cyanobacterial biomass. The study area had not been trampled by livestock or subjected to vehicular or foot traffic for over 40 years prior to treatment.

The study area was chosen for uniformity of vegetation, topography and crustal cover. Treatments were replicated twice and applied randomly within the 3 blocks. Treatments included control plots, shallow raking (surface 2 cm), deep raking (surface 10 cm), scalping away the top 1.0 cm of soil, driving over plots with a tracked vehicle (a 50 ton mobile howitzer with an average ground pressure of an estimated 0.76 kg cm^{-2}) 1, 4, and 10 times, and scalping plus howitzer traffic (4 passes).

Nitrogenase activity and chlorophyll content were analyzed in April, 1992, approximately 9 months after the plots were treated. Samples were collected dry. For nitrogen fixation, 20 samples were collected from each treatment. Samples were collected in 2.5 cm diameter transparent, plexiglass tubes

that were open at both ends. Tubes were pushed into the soil surface to isolate an intact sample. Samples were immediately corked at both ends and transported to a mobile laboratory in upright position. The entire sample was wetted with a constant volume of distilled water on arrival at the laboratory and injected with enough acetylene to create a 10% acetylene atmosphere. After injection, the crust samples were incubated for 4 hours at 26 C in a chamber lighted with Chromo50 (5000 K) and cool white fluorescent bulbs. Subsamples (0.25 mL) of the atmosphere from head space above samples in the tubes were analyzed for concentrations of acetylene and ethylene using a Carle FID gas chromatograph equipped with a 244 cm, 8% NaCl on alumina column, using helium as the carrier gas (30 mL min^{-1}). Results are reported in gas chromatograph units, as conversion to absolute values of N requires calibration with N^{15}.

For chlorophyll measurements, 15, 1 cm deep, samples per treatment were collected dry in 16 mm test tubes and extracted immediately. Techniques outlined in Ronen and Galun (1984) were used. Chlorophyll was extracted from samples with dimethylsulfoxide (DMSO) in the dark for 45 minutes at 65° C. Samples were then centrifuged and absorption spectra were measured for the supernatant liquid with a Hewlett-Packard diode array spectrophotometer previously calibrated with a DMSO blank. Absorbance characteristics of both acidified (using IN HCl) and nonacidified extracts were determined by scanning between 700 and 400 nm. Acidified extracts of the cyanobacterial soil crusts showed a drop at 398 nm (chlorophyll a), and a new peak at 362 nm (phaeophytin), and a drop at 665 nm (chlorophyll a and phaeophytin). Consequently, readings at 362 nm and 398 nm were used to estimate chlorophyll degradation ratios. Readings at 665 nm (chlorophyll a) before and after acidification (with 1 N HCl), and at 750 for turbidity were used to estimate chlorophyll a per unit surface area. Chlorophyll a was determined using an equation modified to express pigment content on the basis of surface area:

$$\mu \text{ chl a cm}^{-2} = \frac{26.73\ (*)(v)}{(A)\ (L)}$$

where (*) is the difference is absorbance before and after acidification (of 665 nm - 750 nm); (v) is the extract volume in ml; (A) is the surface area of the sample in cm^2; and (L) is the path length of the

spectrophotometer cuvette in cm. This equation accounts for any phaeophytin or turbidity in the sample (Beymer and Klopatek, 1992). Chlorophyll degradation ratios were constructed by dividing readings at 398 nm by readings at 362 nm. Results were analyzed using analysis of variance (ANOVA). Where significant differences were demonstrated by ANOVA, Duncan's multiple range test was used to distinguish treatments from each other. A probability level less than or equal to 0.05 was considered statistically significant.

Results and Discussion

Chlorophyll contents were not significantly different between the undisturbed control and treatments that left the disrupted crusts in place. These included the shallow raked, deep-raked, one-pass or ten-pass treatments (Fig. 1). Treatments that involved the removal of crustal material, scalped and scalped with four passes, did show significantly lower chlorophyll contents than the other treatments and the control. An exception to this was the four pass treatment, which showed lowered chlorophyll levels as well. It is not clear why this was observed, and may have been an artifact of sampling. Chlorophyll degradation showed no significant differences among treatments (Fig. 2).

The dominant species found in the crusts, Microcoleus vaginatus, is fairly tolerant of surface disturbances and burial, being capable of moving up to 5 cm in 24 hours when wetted (Susan Campbell, personal communication). This enables this species to reach photic zones even when covered by water- or wind-borne sediments. Given that this cyanobacterium represented the bulk of the chlorophyll in these crusts, it is not surprising that short-term surface disturbances that left the crushed crusts in place did not significantly reduce chlorophyll levels. Scalped plots, where the biotic crust was removed, would be expected to have lower chlorophyll contents until enough time had passed for the photosynthetic microbial populations to re-establish themselves. Since these organisms are only active when wet, recovery time for chlorophyll content of soil is heavily dependent on precipitation, as well as the extent of disturbance.

Effects of treatments on nitrogenase activity, ranked according to activity level, is shown in

Figure 3. The undisturbed control showed significantly higher nitrogenase activity than any treatment. All treatments showed a 77-97% reduction in nitrogenase activity due to disturbance of the biotic crust, with a mean reduction of 88%. Treatment means did not differ significantly from each other.

Several nitrogen-fixing lichen and cyanobacterial species were present in these crusts before disturbance. The lichen Collema is known to be an active fixer (Belnap, 1992). However, brittle thalli and slow growth rates result in this species being easily extirpated when trampled or buried by sediments. Consequently, most surface disturbances would result in a reduction of this lichen's ability to contribute fixed nitrogen to surrounding soils.

Several known nitrogen fixing cyanobacteria were present in the undisturbed crusts, including M. vaginatus, Nostoc sp. and Scytonema myochorus. However, only M. vaginatus was present in sufficient numbers to contribute significant amount of fixed nitrogen. Since nitrogen fixation is an anaerobic process, and this cyanobacterium does not have heterocysts (thick walled cells that exclude oxygen), anaerobic micro-environments must be created in other ways by this species. It is possible this is accomplished by packing of multiple filaments within thick extracellular sheaths (Fig. 4), or by packing groups of sheaths together. This packing phenomenon has been shown for several oceanic cyanobacteria, including the morphologically similar species Microcoleus chthonoplastes (Paerl 1885, 1990; Paerl and Bebout 1988; Paerl and Bland 1982; Paerl and Prufert 1987; Paerl et al. 1989a,b, 1991; Pearson 1981). If this is the mechanism employed by M. vaginatus for oxygen exclusion, then virtually any disruption of tightly packed filaments could create an oxygenated environment and stop nitrogen fixation. Given the extreme brittleness of the sheaths and cyanobacterial mats when dry, virtually any surface disturbances would result in a cessation of nitrogen fixation in this species.

This study demonstrates that recovery time for nitrogenase activity is much longer than for chlorophyll levels and that nitrogenase activity is still greatly suppressed 9 months after the disturbance. This has major implications for studies attempting to assess recovery rates of crusts, where traditional methods have employed visual assessments or chlorophyll levels. In addition, this study shows clearly

that nitrogen fixation is not solely determined by the number of cyanobacterial cells present. If nitrogenase activity in M. vaginatus is dependent in some way on packing of its filaments, recovery of N-fixation activity may be more closely related to the production of sheath material and/or new filaments. Sheath material is produced whenever this cyanobacterium is wetted and its filaments extruded (J. Belnap, personal observation), but there is no information on the rates of production, or factors that control it. In addition, nothing is known about factors that control the growth rate of trichomes, or what determines the number of trichomes per sheath. It has been noted that the number of filaments per sheath tends to be higher in gypsiferous and limestone soils, as well as in sandy soils that have not been subjected to trampling by livestock or humans (Belnap, personal observation).

This study raises many questions critical to management of semi-arid landscapes. These ecosystems naturally maintain little nitrogen in readily available forms, and any activities that adversely affect availability of this often-limiting element may affect the productivity and long-term health of all living components of the system. Research is needed to understand the mechanisms controlling nitrogenase activity in cryptobiotic crusts and how mechanical disturbance affects that activity. With such information available, managers could control actions that damage cryptobiotic crusts and implement actions to hasten their recovery.

Acknowledgments

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Figure 1. Chlorophyll contents (μg cm^{-2}) for the treatments listed. First group of means differs significantly from the second group ($p < 0.05$). Means within groups do not differ significantly.

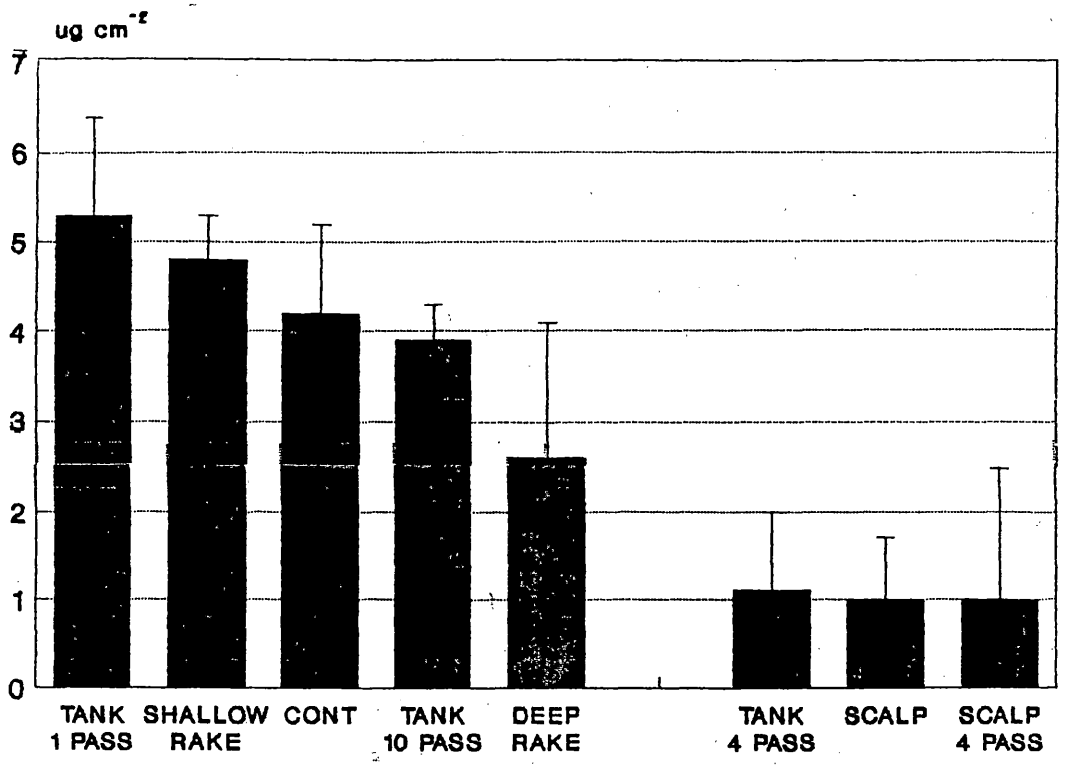

ug cm⁻²
7
6
5
4
3
2
1
0
TANK 1 PASS
SHALLOW RAKE
CONT
TANK 10 PASS
DEEP RAKE
TANK 4 PASS
SCALP
SCALP 4 PASS

Figure 2. Chlorophyll degradation ratios for the treatments listed. No significant differences were found among mean chlorophyll degradation ratios.

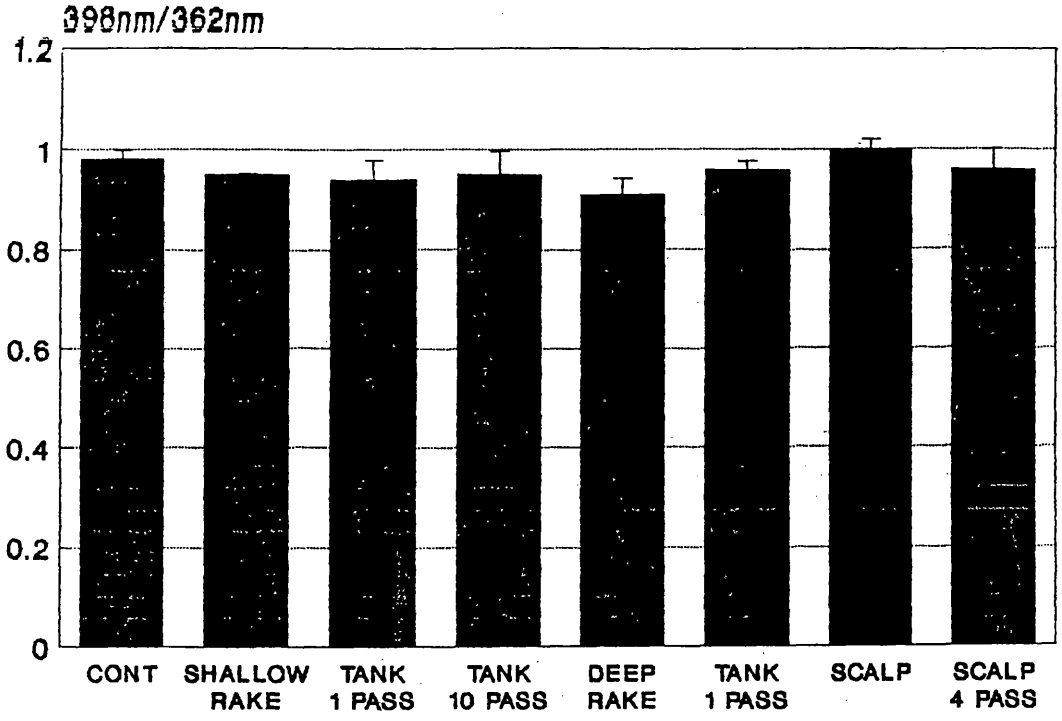
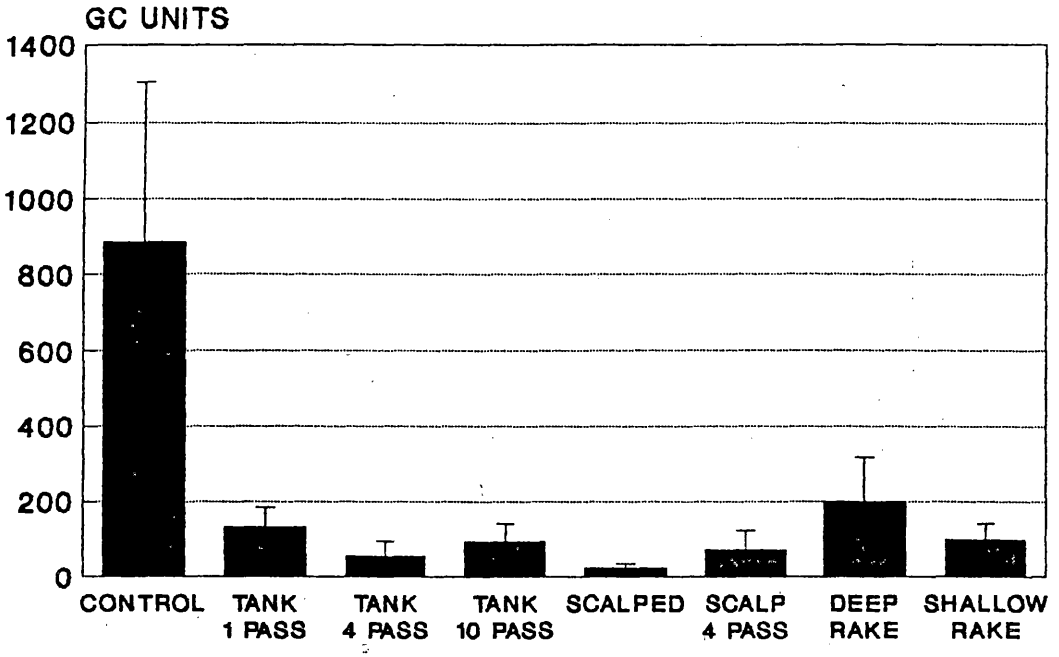

398nm/362nm
1.2
1
0.8
0.6
0.4
0.2
0
CONT
SHALLOW RAKE
TANK 1 PASS
TANK 10 PASS
DEEP RAKE
TANK 1 PASS
SCALP
SCALP 4 PASS

Figure 3. Nitrogenase activity in surface soils that received treatments 9 months earlier. Nitrogenase activity of control was significantly greater than that for all other treatments ($p < 0.02$). Means of treatments did not differ significantly from each other.

SILTY LOAM ALLUVIUM



DUGWAY 3/92; 9 MOS; <77-97%; CONT.**REST

EFFECTS OF COMPRESSIONAL SURFACE DISTURBANCES
PART II: CANYONLANDS NATIONAL PARK

Nitrogen Input in Cold Desert Systems: Effects of Surface Disturbance on Nitrogenase Activity in Soils

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Key words: cryptobiotic crusts, deserts, disturbance, nitrogen fixation, nutrient cycling

Abstract. Crytobiotic soil crusts are an important source of nitrogen for cold-desert ecosystems. Effects of surface disturbance on the nitrogenase activity in crusts dominated by the cyanobacteria Microcoleus vaginatus, Scytonema myochorus and the soil lichen Collema tenax was investigated. Experiments showed that disruptive surface disturbances reduced nitrogenase activity by 30-100%, depending on the type of disturbance. Crusts dominated by the cyanobacterium M. vaginatus on sandy soils were the most susceptible to disruption, with crusts on gypsiferous soils and crusts with larger amounts of C. tenax less suceptible to disruption. Reasons for this differential reduction are discussed, as well as the implications for nitrogen budgets in cold-desert ecosystems.

Introduction

Nitrogen levels are known to be low in desert systems relative to other systems. Total atmospheric input over the past 10 000 years has been conservatively estimated to be only 2.99 kg N/m^2, with 77% of this lost through wind erosion, ammonia volatization, nitrification, and denitrification (Peterjohn & Schlesinger 1990). Among deserts, cold deserts are expected to have even lower nitrogen levels, as extensive surveys have revealed only a few nitrogen-fixing plants, or rhizospheres, in these regions (Wullstein 1989; Farnsworth et al. 1976) and denitrification levels are high (West and Skujins 1978). In addition, concentration of nitrogen around perennial plants, through litterfall and "mining" of the interspace by plant roots, results in interspaces having even lower levels of essential nutrients. Since nitrogen has been shown to limit net primary productivity in many desert ecosystems (Ettershank et al. 1978; Fisher et al. 1988, Nobel et al. 1988; James & Jurinak 1978; Romney et al. 1978), any biological input is critical to the continuing fertility of these cold-desert regions.

Most cold deserts are dominated by cyanobacterial-lichen soil crusts. These crusts often represent 70% or more of the living cover in these areas, and can be up to 10 cm thick. Many rainfall events in desert areas are not large enough to promote plant growth, but do stimulate microbial community activity. Since cyanobacterial-lichen soil crusts fix nitrogen that has been demonstrated to affect the nutrition of nearby vascular plants (Belnap 1992; Mayland et al. 1966; Mayland & MacIntosh 1966; Belnap & Harper, personal observation), they may be able to supply this nutrient to the system more often than nitrogen-fixing plant species. In addition, the presence of crusts in interspaces between vascular plants enable them to play a critical role in maintaining a more homogenous nitrogen landscape in deserts.

Nitrogen fixation in these soil crusts has been examined on several occasions, with variable results. Nitrogen input estimates from these crusts has ranged from 4 to 365 kg/ha annually (Mayland et al. 1966; MacGregor & Johnson 1971; Rychert & Skujins 1974; Eskew & Ting 1978; Jeffries 1989). Nitrogen fixation in these crusts is highly dependent on past and present water and light regimes, as well as the species composition (Rychert et al. 1978). Timing, extent and type of past disturbance may also be a critical factor in determining fixation rates.

Response to, and recovery from, disturbance has been investigated in these crusts. Response and recovery have traditionally been measured in terms of visual recovery, or biomass or numbers of organisms present, while effects on physiology have not been addressed. A recent study at Dugway Proving Grounds in Utah demonstrated that disturbance does affect the physiological functioning of these crusts: plots receiving surface disturbances ranging from shallow raking of soils to compression by mobile howitzers showed a 77-90% decrease in nitrogenase activity 9 months after the disturbance (Belnap et al. 1993). Since many of the surface soils in the West receive compressional disturbances in the form of human or livestock trampling, as well as off-road vehicle use, this study examines the effects of these types of activities on nitrogenase activity in crusts on sandy and gypsiferous soils.

Materials and Methods

All sites were located with 10 miles of Moab, Utah, at an elevation of approximately 4500'. Three substrates were tested: soils derived from Navajo sandstone, Mancos shale and Paradox gypsum. On sandy soils, Coleogyne ramosissima (blackbrush) was the dominant vascular plant; on both the Mancos shale and gypsiferous soils, the vascular plant flora was dominated by Atriplex confertifolia. The areas chosen for study had well- developed cyanobacterial-lichen soil crusts. The cyanobacterium M. vaginatus was dominant in all the soils tested, often being the only cyanobacterial species present in any given sample. Sandy soil sites were in Arches National Park (north of Moab, Utah) and the Behind-the Rocks (BTR) area (south of Moab, Utah). At Arches, vehicle, bicycle and human tracks were put in two types of crust: those with only M. vaginatus present (light crust) and a second type with M. vaginatus, some S. myochrous and approximately 5% cover of the nitrogen-fixing soil lichen C.tenax (dark crust). At the BTR site, dark crusts were sampled. On the gypsiferous soils, C. tenax represented approximately 25% of the ground cover, with M. vaginatus and S. myochrous both present. Mancos shale crusts were almost entirely made up of M. vaginatus, with no lichens present. Preliminary chlorophyll and nitrogenase assessments at the Mancos sites showed such low cyanobacterial biomass levels and low nitrogenase activity levels that this substrate- type was dropped from further study.

Two sandy and 2 gypsiferous sites received selected treatments. Treatments included 2 and 4 passes with a Toyota Landcruiser; 1 and 2 passes with a knobby-tire mountain bike; 1 and 3 passes with a knobbed, Vibram sole boot; 1 and 3 passes a smooth-soled sandal;

and a small wooden block (15x30 cm) that was stepped on 1 time to compress the soil with the least churning action possible. Not all treatments were repeated at all sites. All soils had less than 1% soil moisture (gravimetrically determined) with the exception of the second gypsum treatment, when soil moisture was 4%.

Nitrogenase activity and chlorophyll content were analyzed before and immediately after treatments were applied; selected treatments were then sampled over time. Since nitrogenase levels naturally fluctuate throughout the year, controls and treatments were run on the same day throughout the experiment. Samples were collected dry. For nitrogen fixation, 20 samples were collected from each treatment. Samples were placed in clear, gas-tight tubes, the entire crustal surface was wetted equally with distilled water, and then injected with enough acetylene to create a 10% acetylene atmosphere. After injection, samples were incubated for 4 hours at 26 C in a chamber lighted with Chromo50 (5000 K) and cool white fluorescent bulbs. Subsamples (0.25 ml) of the head space within the tubes were then analyzed for acetylene and ethylene content on a Carle FID gas chromatography equipped with an 8 foot, 8% NaCl on alumina column, using helium as the carrier gas (30 ml/min).

For chlorophyll measurements, 15 samples per treatment were collected dry, and extracted immediately. The techniques outlined in Ronen and Galun (1984) were used. Chlorophyll was extracted from samples with dimethylsulfoxide (DMSO), in the dark for 45 minutes at 65 C. Samples were shaken, and a subsample then centrifuged. Absorption spectra were measured in a Hewlett-Packard diode array spectrophotometer, after calibration with a DMSO blank. Optical densities used for measurements were at OD 398 (chlorophyll a) and OD 665 (both chlorophyll a and phaeophytin). Results were analyzed using analysis of variance (ANOVA), Duncan's multiple range test, and where only two treatments were applied, an unpaired t-test.

Results

Results from these experiments are summarized in Table 1-3. Since nitrogenase activity is highly dependent on past and current environmental variables, different sites or sample dates can only reliably compared with controls run on the same day. Comparisons of absolute nitrogenase activity levels between sample dates is not possible, but ratios to controls must be used. As can be seen from Tables 1-3, almost all surface disturbances of any type significantly reduced nitrogenase activity in crusts at all sites, although nitrogenase activity of controls remained active throughout the sample period. Cyanobacterial biomass, as measured by chlorophyll a levels, was not significantly different from controls in any of the treatments.

Wheeled Vehicles (Trucks and Mountain Bikes): All sites showed significantly lowered nitrogenase activity when driven across, with no significant difference between one or three passes. Vehicular tracks reduced nitrogenase activity in less well-developed (light) crust at Arches by 86-88%, and in dark crusts by 52-100%. At the

BTR site, reductions were 40-100%. Mountain bike tracks were measured in the light crust at Arches, and reduced activity by 54-73%. At BTR, activity was decreased by 79%. Dark crusts showed less disruption of nitrogenase activity than light crusts when done on the same day. For dark crusts on sandy soils where samples were repeated, early spring measurements (April) showed less disruption than summer and fall measurements (49-52% vs. 97-100%). Tracks put in later in the season showed a tendency to be more disruptive at the BTR site.

Gypsiferous soils showed a similar pattern to sandy sites, although activity differences were less. Three passes with a vehicle resulted in nitrogenase activity reductions of 48% when soils were dry, and 30% when soils had 4% moisture. This site, resampled several weeks later, showed a reduction of 63%.

Footprints: Three types of shoes were used for footprints. These included boots and tennis shoes with lugged soles, and sandals with smooth soles. Experiments were done in dark crust only. Boots at Arches showed a 35-92% decline in activity. When compared with wheeled vehicle treatments on the same day, there were no significant differences. At BTR, the lugged boots and smooth sandals were compared: while the lug soles decreased activity by 79%, and was not significantly different from the wheeled vehicle treatment, smooth soles reduced activity by 50%. The smooth sole treatment was significantly less than the lugged sole or wheeled vehicles.

Gypsiferous soils showed a reduction of 60% when lugged boots were used. No disruption was seen with the smooth-soled sandals. Again, values for boots and vehicles were not significantly different from each other, but were significantly lower than smooth soles.

Wooden Block: This was used in sandy soils at the BTR site. This treatment resulted in a decrease of 25% in nitrogenase activity, the least disruption for any treatment on sandy soils.

Discussion

Most soil crusts in the cold-desert regions of the Colorado Plateau (southern Utah, northern Arizona, and western Colorado) are dominated by the cyanobacterium Microcoleus vaginatus. Well-developed, undisturbed crusts also contain the cyanobacterium Scytonema myochrous and the soil lichen Collema tenax (with the cyanobacterium Nostoc as a phycobiont) as well. M. vaginatus is a filamentous species that occurs as multiple filaments contained within a single, extracellular polysaccharide sheath. This species lacks heterocysts, the structurally differentiated, oxygen-excluding cells where cyanobacterial nitrogen fixation generally takes place. In contrast, S. myochrous and Nostoc are both heterocystic species. All three species are capable of both light and dark nitrogen fixation (Pearson et al. 1981; Paerl 1990; Belnap 1992). Since nitrogen fixation is an anaerobic process, species such as M. vaginatus that lack heterocysts must utilize other methods to exclude oxygen. In oceanic systems, Microcoleus chthonoplastes, morphologically almost identical to the terrestrial

M. vaginatus, create anaerobic microzones through aggregation of filaments, both within and between sheaths (Pearl 1985, 1990). Nitrogenase activity has been demonstrated repeatedly in other non-heterocystous aquatic species, including Aphanizomenon flos-aquae (Carlton & Paerl 1989), Trichodesmium erythraea (Bryceson & Fay 1981), Trichodesmium sp. (Paerl & Bebout 1988; Paerl et al. 1989b; Carpenter & Price 1976), Microcoleus chthonoplastes (Pearson et al. 1981), and Lyngbya asetuarii (Paerl et al. 1991). Separation of the nitrogenase enzyme from oxygen is accomplished spatially, temporally or through a combination of both (Paerl 1990; Paerl and Bland 1982; Paerl and Prufert 1987). Trichodesmium, Microcoleus chthonoplastes and Aphanizomenon flos-aquae have been shown to create anaerobic microzones spatially through aggregation both between and within sheaths. These species all showed reduced nitrogenase activity when shaken or when aggregates were disrupted (Paerl 1990). Bacteria associated with these species may be partially responsible for anaerobic microzone formation through the scavenging of oxygen or may contribute N through their own fixation (Paerl et al. 1989a). Scanning electron microscope observations of soil crusts (Belnap & Gardner 1993) show that M. vaginatus could employ any or all of the strategies used by aquatic species, since it has multiple trichomes within a single sheath, packing of multiple sheaths on top of each other and associated bacteria that may be scavenging oxygen.

Previous studies have shown that many types of disturbances negatively affect the cohesion and coverage of soil cyanobacterial-lichen crusts (Belnap & Gardner 1993; Cole 1991; Wilshire 1983). The most common disturbance to these crusts is compressional forces created by livestock, human feet or off-road vehicles. Cyanobacterial filaments and lichens are brittle when dry and easily crushed by these compressional forces (Webb & Wilshire, 1983; Harper & Marble 1990; Belnap 1993). These types of disturbance would have the potential of disrupting cyanobacterial aggregations. If anaerobic microzone formation is dependent on filament and sheath aggregation for Microcoleus in soils as it is in water, surface disturbances may reduce or eliminate the capability of these organisms to fix nitrogen.

Results from this study support the hypothesis the aggregation is a major factor in the formation of microzones for M. vaginatus. Since there was no difference in chlorophyll concentration among the treatments or the control, the large differences seen in nitrogenase activity between treatments cannot be explained by differences in numbers of cyanobacteria present. If microzone formation was dependent only on bacterial scavenging, much less disruption of fixation would be expected from a single footprint than was seen in this study, and there little difference would be expected in fixation levels between the types of treatments used this experiment. Aggregations of cyanobacteria, however, would be disrupted by any surface disturbance, with more disruptive disturbances resulting in greater reduction of nitrogenase activity. Lugged surfaces that break or churn the surface, such as vehicle tires or Vibram soles on boots, were more disruptive than smooth-soled sandals or wooden blocks that tend to only compress the soil. Greater amounts of compressional forces involved did not

result in greater nitrogenase activity reduction: bikes and Vibram soles were just as damaging as a vehicle. Similar results were seen in the study done on fine-textured lacustrine soils at Dugway Proving Grounds. Treatments included shallow and deep raking; scalping of the top 1 cm of soil; and 1, 4 and 10 passes of a mobile howitzer (a tracked vehicle). All were highly disruptive of the surface, and resulted in a 77-90% reduction in nitrogenase activity. However, differences in compressional force did not result in any differences between the treatments.

Relative to controls, dark crusts showed less disruption in nitrogenase activity than light crusts. This could be explained by the species present. Light crust is almost exclusively non-heterocystic Microcoleus sp., and therefore would be highly sensitive to any surface disturbance that would disrupt aggregations. Dark crusts contain heterocystic species such as Scytonema and Collema, making this crust somewhat less dependent on aggregation for nitrogenase activity.

Greater reduction of nitrogenase activity could be expected over time in disturbed sites, as was seen at both sites where this was measured (Arches and BTR). Disruption of the soil surface would immediately affect only M. vaginatus. However, pieces of lichen thalli buried by the disturbance would die over time; also, many disconnected pieces could be blown or washed away. Over time, nitrogen contributions from these sources would decline. In addition, timing of disturbance may affect reduction rates. Since all these organisms are only metabolically active when wet, recovery depends on moisture. Consequently, disturbance to surfaces in drier and hotter times would be expected to result in greater impact over time to these sites.

Gypsiferous soils showed less reduction in nitrogenase activity when compared to sandstone-derived soils. There are two possible explanations for this. First, dry gypsiferous soils are more cohesive and have higher structural integrity than dry sandy soils, and would therefore be more resistant to disruption. Gypsum is highly soluble in water, and particles adhere to each other upon drying, instead of being loose like grains of sand. When stepped on, gypsiferous surfaces offer much more resistance than sandy substrates. Therefore, "packing" of the crust would more likely be preserved in a gypsiferous soil. Secondly, there is much more of the lichen C. tenax on gypsiferous crusts, occurring as scattered small dots throughout the crust. Disruption of packing would probably not result in a short-term reduction of nitrogenase activity.

This study has many implications for ecosystems which are dependent on these crusts for nitrogen. Since many of the cold-desert systems in which these crusts occur have few, if any, nitrogen-fixing plants, surface disturbances (such as trampling by livestock, people and/or recreational vehicles) could result in dramatic decreases in nitrogen input from these crusts and would consequently have ecosystem-wide effects. Results from a study done by Belnap and Harper (unpublished data) show that plants growing in uncrusted areas have significantly lower nitrogen content than plants growing in well-developed crusts. Evans and Ehlringer (1993) showed that crusts were the predominant source of

nitrogen for the two juniper woodland sites measured. Since these cold-deserts have been grazed extensively for 100 years, and now are receiving ever-increasing recreational uses, understanding of the relationship between surface disturbances and nitrogen cycles is critical.

This study also forces a re-examination of the definition of recovery for these soil crusts. Traditional recovery assessment using visual estimates or using estimations of biomass may not be adequate, as recovery of the physiological functioning of these crusts needs to be included as well. Only one aspect of their physiology, nitrogenase activity, has been examined relative to disturbance. There may be other physiological aspects that are disrupted as well. Until such time that further studies can be done, and recovery time from such disturbance can be better estimated, it is important that these resources be managed conservatively.

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Table 1. Arches site: Percent decline of nitrogenase activity with different surface disturbances at 6 sampling dates. All declines were significantly different from controls, but not different among treatments.

DATE SAMPLED	DATE TREATED	CRUST TYPE	VEHICLE 1 PASS	VEHICLE 3 PASSES	BIKE 1 PASS	BIKE 3 PASSES	BOOT 1 PASS	BOOT 3 PASSES
4/14/92	4/14/92	L	88%		54%	73%		
4/28/92	4/14/92	L	86%	85%				
4/14/92	4/14/92	D	52%	66%			60%	92%
4/28/92	4/28/92	D					45%	32%
4/28/92	4/14/92	D	49%					
6/2/92	4/14/92	D	98%	95%				
6/13/92	4/14/92	D	97%	100%				
10/20/92	4/14/92	D	100%					

Table 2. Behind-the Rocks (BTR) site: Percent decline of nitrogenase activity with different surface disturbances at 8 sampling dates. All declines were significantly different from controls. Sandal and wood treatments were significantly lower than other treatments, and not different from each other.

DATE SAMPLED	DATE TREATED	VEHICLE 1 PASS	BIKE 1 PASS	BOOT 1 PASS	TENNIE 1 PASS	SANDAL 1 PASS	WOOD DRY
6/4/92	6/4/92	69%					
6/6/92	6/6/92		79%	79%		50%	
6/7/92	6/7/92			62%		38%	25%
9/30/92	9/30/92			61%	81%		
10/5/92	10/5/92	92%		85%	96%		
10/19/92	6/4/92	100%					
10/19/92	10/19/92			50%	85%		
10/25/92	10/25/92	91%					
11/30/92	11/30/92	40%		45%			

Table 3. Gypsum site: Percent decline of nitrogenase activity with different surface disturbances at 2 sampling dates. Vehicles and boot treatments were significantly different from the control; sandal treatment. There was no difference between the sandal treatment and the control.

DATE SAMPLED	DATE TREATED	VEHICLE 3 PASSES	BOOT 1 PASS	SANDAL 1 PASS
10/18/92	10/18/92	48%	60%	0%
11/4/92	11/4/92	63%		
11/10/92	11/10/92	30%		0%

MICROBIAL ANALYSIS OF REVEGETATION PLOTS IN CANYONLANDS NATIONAL PARK

MICROBIAL ANALYSIS OF REVEGETATION PLOTS IN CANYONLANDS NATIONAL PARK

Revegetation efforts in semi-arid areas have met with limited success. Harsh growing conditions, arid climate, and soil composition make successful restoration of vegetation communities a difficult task. Additionally, the existing body of restoration literature on these lands is virtually non-existent. Testing of different methods and techniques both in the laboratory and in the field is needed. For this reason, an extensive revegetation research project was undertaken at Canyonlands National Park. This project was designed to test the effects of different soil treatments on germination and establishment of native plant communities in disturbed arid land sites.

The project was begun in Fall, 1991, when sites were selected, treatments applied, and areas seeded with the native dominant grass Stipa (Oryzopsis) hymenoides (for details of project and explanation of the different treatments, see the first report, "Revegetation of Disturbed Semi-Arid Grassland in Canyonlands National Park). In Fall, 1992, treatment effects were evaluated by quantifying the number of seeded plants that had established. Analysis of results indicated that plots were dominated by two species, the seeded native perennial Stipa and the annual exotic Salsola.

Large, and statistically significant, differences were detected in the success of the native perennial grass. Below-ground food web structure and function can strongly influence above-ground vegetative community structure, especially the establishment of perennials versus annuals. For this reason, it was decided to see if differing levels of Stipa and Salsola establishment were correlated with differences in microbial populations. Analysis including VAM infection rates, total and active bacterial biomass, total and active fungal biomass and total nematode numbers (divided into major functional groups) was done for selected treatments.

Attached are two reports resulting from this work. The first report, "Revegetation of Disturbed Semi-Arid Grassland in Canyonlands National Park" describes the overall revegetation project, including the treatments applied, the assessments of Stipa success, and VAM infection rates of Stipa for the different treatments. The second report, "Bacterial/Fungal Analysis of Revegetation Plots in Canyonlands National Park" addresses the results from the analysis of active and total fungal and bacterial biomass. Results presented in this report are preliminary, as the contracted sample analyses are not yet completed. Nematode analyses, as well as finalized versions of the bacteria and fungal biomass, will not be available until November, at which time a formal manuscript will be prepared with this data. However, several important conclusions can be reached from the available data, and are discussed.

MICROBIAL ANALYSIS OF REVEGETATION PLOTS IN CANYONLANDS NATIONAL PARK
PART I: VESICULAR-ARBUSCULAR MYCORRHIZAE

Revegetation of Disturbed Semiarid Grassland in Canyonlands National Park

INTRODUCTION

The southwestern portion of the United States is characterized in part by arid and semiarid deserts. Many of these landscapes have been severely overgrazed resulting in denuded soil surfaces and heterogeneity of soil resources (Schlesinger et al. 1990). Disturbance in arid climates may be followed by shifts in species composition as a result of reduced organic matter and soil moisture, and increased soil temperatures from loss of shade (Allen and MacMahon 1985). Re-establishment of vegetation on disturbed southwestern desert lands has been mildly successful, the resulting vegetation typically differing in composition, and many times not persisting to establishment (Thornburg and Fuchs 1978); the level of mycorrhizal fungi remaining in the soil appears to be an indicator of the type and rate of plant succession that will follow (Doerr et al. 1984). Failures in semiarid revegetation may be due in part to the focus on plant species to the exclusion of soil patterns and processes, plant-soil relationships, and the functioning of the ecosystem as a whole.

Revegetation research on disturbed arid lands has focused almost exclusively on mined lands, though 245 million hectares (27%) of land in the United States consists of rangeland pasture compared to 1.5 million hectares (.2%) of mined lands (Paone et al. 1978). Regardless of the cause

of disturbance, large areas of semiarid lands have lost their functional stability and are in need of rehabilitation (Box 1978). The systems and processes which create stability in semiarid deserts are not well understood.

Soil disturbance is a significant barrier to regeneration in semi-arid environments due at least in part to the fragile and easily damaged soil crusts which characterize these systems (Campbell et al. 1989, Dunne 1989). Cryptobiotic soil crusts, formed and held together primarily by cyanobacteria, lichens, mosses, bacteria and fungi in the southeastern Utah desert, are critical to soil stability, nutrient and water retention, water infiltration, and nitrogen fixation (Beymer and Klopatek 1992, Anderson et al. 1982 (1), Brady 1974, St. Clair et al. 1984, and MacGregor and Johnson 1971, Isichei 1990). Water absorption capacity and nutrient retention are particularly important in the semiarid West where precipitation and minerals, phosphate in particular, are limiting to plant growth (Williams and Aldon 1976). Disturbance to these crusts affects plant establishment and revegetation (Doerr et al. 1984). Soil crusts seem to be an appropriate area of focus for restoration, if taken in the context of the soil-plant connection, the effects on the rate of mycorrhizal colonization as a result of crust formation, and the resultant response of the host plants.

Most semi-arid grassland plants form vesicular-arbuscular mycorrhizae (VAM) (Trappe 1981). This symbiosis has been found to benefit both individual plants and ecosystem processes (Anderson 1987, Reeves 1987). VAM form a hyphal network through which material may be transferred between individuals of the same and different species in a community, which in turn stabilizes ecosystem nutrient fluxes (McNaughton and Oesterheld 1990, Perry et al 1992). Some of the functions which have been found to benefit VA mycotrophic as compared to nonmycotrophic plants include; increased absorption of water and nutrients, drought resistance, pathogen protection, maintenance of CO_2 uptake, reduced water loss, better absorption of mineral ions and increased plant growth (Molina and Amaranthus 1990, Huang et al. 1975, Miller 1987, Allen 1991). Plants in semiarid grassland communities are particularly influenced by VAM associations (Miller 1987).

The presence and distribution of mycorrhizal plants has been related to water availability, successional stages within a community, and mycorrhizal inoculum potential in the soil (Pendleton and Smith 1983). Mycorrhizal plants are more typical of undisturbed and later seral stages of semiarid habitats (Reeves et al. 1979). The establishment phase following disturbance is an important determinant of plant community due to the few seral stages typical of semiarid ecosystems (Kleiner 1983, Fowler 1986). Soil

disturbance has been found to reduce mycorrhizal inoculum potential (Warner et al. 1987) and favor nonmycorrhizal plants on semiarid disturbed land (Reeves et al. 1979, Allen and Allen 1987, El-Tayeb and Skujins 1989). When infected, mycorrhizal species have been found typically to establish more quickly than nonmycorrhizal species due to higher survival rates and greater production (Doerr et al. 1984).

This research is part of a larger project designed and implemented by Canyonlands National Park under the direction of Dr. Jayne Belnap. I participated in setting up the research plots, seeding and applying soil treatments, and measurement of seedling establishment and mycorrhizal colonization. This paper describes my part in the study, examining revegetation of disturbed soils with native grasses and their subsequent relative colonization of VAM.

The objectives of the study were to determine the affects of various soil treatments on 1) Stipa seedling establishment, and 2) relative colonization of VAM on Stipa. Two Stipa species were seeded and various soil amendments were applied. Relative VAM colonization of Stipa and Stipa density were measured.

Hypotheses and Rationale

Stipa Density

Hypothesis 1. Cryptobiotic soil crusts, sugar, straw mulch, hay and water will each increase Stipa establishment in

comparison to the control treatment of seeding only plus water.

Rationale. Topsoiling has been found to enhance plant establishment and growth in revegetation of semiarid mined sites (Packer and Aldon 1978). The presence of soil biota and organic matter may improve soil structure and plant growth (Forster 1990). Inoculation with healthy crusts may thus speed crust formation and the retentive functions the crusts provide. Sugar should affect nutrient availability through a chain-reaction series of responses; increased microbial activity, decreased available nitrogen to the annuals, greater establishment of perennials which have lower nutrient requirements (McLendon and Redente 1991). Straw mulch shades the plants which should reduce soil temperature, moisture loss and heat stress (Packer and Aldon 1978). Hay was added as a relatively low C/N source of organic matter that should improve soil water-holding capacity and provide aeration, reducing compaction of soil Water should affect the ability of plants to use nutrients and thus encourage growth (Ries and Day 1978).

Hypothesis 2. Fertilizer will decrease Stipa establishment in comparison to the control treatment.

Rationale. Fertilizer increases available nutrients which tends to increase overall plant growth, though may encourage annual weeds which reduces nutrient availability for initial establishment of perennial grasses.

VAM

VAM formation should develop with the availability of root density (Allen 1992) and carbohydrates from the plants. Extent of colonization is typically correlated with plant growth (Harley and Smith 1983). Therefore, I expect colonization to increase with the same amendments as for Stipa density. Additional reasons for development and discouragement without regard for Stipa density are listed below.

Hypothesis 3. Cryptobiotic soil crusts, sugar, straw mulch, hay and water will each increase relative VAM colonization in comparison to the control treatment of seeding only plus water.

Rationale. The soil stabilization created by the cryptobiotic crusts may provide a more secure surface for mycorrhizal inoculum to locate roots for formation. Sugar should stimulate biololgical activity, including bacteria which stimulate mycorrhizal formation. Organic amendments have also been found to increase mycorrhizal development (Johnson and McGraw 1988). Mulch has been found to encourage VAM formation where nitrogen is adequate and phosphorus limiting (anecdotal observation, Wilson and Wilson 1992). Soil aeration (hay) and soil moisture have been associated with higher mycorrhizal inoculum potential (Allen 1992).

Hypothesis 4. Fertilizer will decrease relative VAM colonization in relation to the control treatment.

Rationale. Mycorrhizal abundance has been found to decrease as soil fertility increased in a natural ecosystem (McNaughton and Oesterheld 1990). High levels of inorganic fertilizers used for reclamation often have also been found to inhibit mycorrhizal formation (Allen 1992).

METHODS

Study Area

The study area, in Canyonlands National Park, Utah, is in a cold desert at 1370 m elevation characterized by seasonality of temperature and precipitation. Temperatures have an annual range of 55°C. Yearly precipitation of 32 cm mainly occurs from March to October, the highest months being July and August (Needles District Visitor Center records, Canyonlands National Park). The site is relatively flat with a general soil texture of 70% sand, 21% silt, 9% clay, and a pH of 8 (Loope 1978).

The study site is a 1.4-acre strip parallelling a paved road, severely disturbed due to construction in the Needles District of Canyonlands National Park. Prior to recent disturbance, the site supported two seeded grasses, Oryzopsis hymenoides and Stipa comata. Other pre-disturbance species include Aristida purpurea, Helianthus annua, Sphaeralacea coccinea, Ambrosia acanthicarpa, Stephanomeria exigua, Atriplex canescens, Gutierrezia sarothrae, Heterotheca villosa, Chrysothamnus nauseousus, Artemisia tridentata, Hilaria jamesii, Astragalus mollissimus, Aster machaerantha, Mentzelia cronquistii, Cycloloma striplicifolia, Salsola kali, and Bromus tectorum.

The area was moderately grazed from the mid-19th century until early 1970's when creation of the National Park allowed

removal of cattle (Kleiner 1983). The area was consequently disturbed from overgrazing prior to the recent disturbance. The aim of the final product of the revegetated area is an imitation of an area of the park which was never grazed, and is dominated by native grasses and forbs with a minimal shrub component.

Soil Treatments and Rationale

The study site was divided into 126, 20.90 m^2 plots representing eighteen different treatments each replicated seven times in a randomized block design. The treatments are listed in Table 1 followed by the rationale.

Table 1. Soil treatments applied to 126, 20.90 m^2 plots replicated seven times in a randomized block design.

Treatment
Treatments with water:
T1: Cryptobiotic soil crusts, applied fall.
T2: Cryptobiotic soil crusts and sugar, applied fall.
T3: Cryptobiotic soil crusts, applied fall, and fertilizer, applied spring.
T4: Cryptobiotic soil crusts, applied fall, and straw mulch, applied spring.
T5: Cryptobiotic soil crusts, applied fall, fertilizer and straw mulch, applied spring.

T6: Cryptobiotic soil crusts and sugar, applied fall, straw mulch, applied spring.

T7: Cryptobiotic soil crusts and wheat hay, applied fall.

T8: Sugar, applied fall, and straw mulch, applied spring.

T9: Straw mulch and fertilizer, applied spring.

T10: Straw mulch, applied spring.

T11: Cryptobiotic soil crusts, applied spring.

T12: Cryptobiotic soil crusts and sugar, applied spring.

T13: Cryptobiotic soil crusts and fertilizer, applied spring.

T14: Cryptobiotic soil crusts and straw mulch, applied spring.

T15: Fertilizer, applied spring.

T16: Control: seed only.

Treatments without water:

T17: Seed, no water.

T18: Cryptobiotic soil crusts and sugar, applied spring, no water.

Rationale

Twelve treatments involved the application of cryptobiotic crusts to the soil surface in either the fall or the spring. Crusts were added to speed natural crust formation, stabilize soils from erosion, retain water and nutrients, and enhance water infiltration (Anderson et al. 1982 (1 & 2), Beymer and Klopatek 1992). Eleven of the 12 crust treatments were irrigated, and were combined with one of the following additional treatments: (a) none (crusts only)

(b) sugar spread on the soil surface (c) fertilizer (1:2:1 nitrogen, phosphorus, potash) (d) straw Hilaria mulch applied to the soil surface, or on top of the soil crusts in treatments with crusts, and (e) wheat hay mixed into the soil.

Amendments tested in isolation were compared to the control treatment of seed only plus water (T16) for analysis. To test the effects of sugar and hay comparisons were made of combinations of each with another amendment to the other amendment alone. The comparisons are listed in Table 3-1 and 3-2 in the Results section.

Procedures

The project began in May of 1991. Seeds of the Stipa and Oryzopsis species were collected in the Park during the summer of 1991 within two miles of the study site. The herbicide Roundup was applied to the corridor with a mechanical sprayer in June and August as an initial attempt to control the dominance of Salsola kali, a pervasive exotic in disturbed areas of the West (Beatley 1973). The intended rate of application was 3.5 L/ha, though the June application was erroneously put out at 28 L/ha, eight times the intended rate. The August application was applied at the original rate and resulted in 2.2 kg total. Following herbicide treatment, the corridor was turned with a tractor disk to a depth of 20.

Seeding was done in the fall to take advantage of accumulated moisture of the summer rains (Plummer 1977).

Seeding began in October of 1991. Oryzopsis hymenoides was drill-seeded at an average depth of 7.5 cm to avoid losing seed to herbivory (SCS 1988, Fulbright et al. 1982). Oryzopsis hymenoides was drilled at the rate of 215 seeds/m^2 resulting in 8.2 kg total seed drilled. Oryzopsis hymenoides was then hand broadcast at the rate of 54 seeds/m^2, in addition to the drilling to avoid visual rows of grass resulting from the lines the tractor seeded. Stipa comata was hand broadcast at the rate of 110 seeds/m^2, except for the first 23 plots of the corridor (all treatments of the first replicate and treatments 3, 4, 11, 13 and 14 of the second replicate) where it was erroneously seeded at 160 seeds/m^2. Stipa comata was not drill seeded because germination typically occurs the first season after planting (SCS 1988), and seed was not subject to herbivory for as long a period of time as Oryzopsis hymenoides. The entire corridor was hand-raked to cover seed.

All fall treatments to the corridor occurred during October and November of 1991. Fall soil treatments were applied in the order of hay, sugar and cryptobiotic soil crusts. Sugar was hand-distributed on top of the soil at a rate of 660 kg/ha. Hay was mixed into the soil 15 cm deep and applied at a rate of 1/2-bale per plot.

Cryptobiotic soil crusts were removed from a site slated for future disturbance, within 1 km of the study site, and re-applied within hours. Vegetation on the site where soils

were collected was similar to the mixed grassland/shrubland adjacent to the study site described under Study Area. The Stipa and Oryzopsis species seeded were inclusive of this community, in addition to other native grasses and forbs. Some exotic species existed there, though there was no evidence of Salsola kali, the most noxious of the local weeds, and the non-native species that did exist were a minor part of this community. The top 15 cm of soil were shovelled into a pile on the bed of a pickup truck. The top 5 cm consisted of cryptobiotic crusts, while the remaining 10 cm was the underlying topsoil, included for ease of spread. This depth should have included the mycorrhizal fungi thought to be confined to the rooting zone of vegetation (Safir 1987), the surface 20 cm in semiarid ecosystems (Sparling and Tinker 1978). The mixture was scooped into 19-liter buckets with four buckets applied to each plot. This resulted in a 1:3 mixture of crusts to topsoil. 2.3 kg of crusts and 6.8 kg of topsoil were broadcast across the surface for a total of 9.1 kg crusts and soil per plot.

Spring soil treatments of sugar, fertilizer, and cryptobiotic crusts were applied in March of 1992 followed by mulch in May. Sugar was applied by hand as during fall treatments. Fertilizer was applied in May for all inclusive treatments, fall and spring alike. Spring application was done to avoid loss of nutrients with precipitation. 1.1 kg of fertilizer was hand spread across each plot. The fertilizer

consisted of a 1:2:1 ratio of nitrogen, phosphorus and potash. Spring cryptobiotic soil crusts were collected from the same area and applied to plots in a similar manner as in the fall. The mulch consisted of straw Hilaria jamesii and was spread on top of crusts to a depth of 5 cm for 90% coverage. Two to three bales per plot were used to accomplish this coverage.

Sugar treatments were re-applied in March, May, July and September of 1992 and will continue every other month between March and November throughout the 3-year study period.

Irrigation was initiated at the beginning of July 1992 and will continue monthly contingent upon rainfall in comparison to a 90-yr monthly precipitation average of 8.5 cm. The amount of water released bi-monthly is figured to equal the 90-yr average. One-half cm of water is released in a 10-minute watering event.

Measurement

Measurement consisted of counting Stipa stems, and determining the colonization rate and genera of vesicular-arbuscular mycorrhizae on the Stipa sp. Stipa density was used as the indicator of establishment. Aseptate mycorrhizal hyphae and VAM vesicles were examined to determine colonization rate while spores were sieved from soil samples to identify VAM genera.

Stipa Establishment

Stipa stems were counted in July and September of 1992. Plants were counted in 5 random square meter quadrats chosen from a 16-m^2 quadrat within each plot. On all plots, Stipa stems were counted in the same quadrats within each of the July and September sampling periods.

Vesicular-arbuscular Mycorrhizae

In June of 1992, five samples of Stipa sp. roots were excavated from each plot for determination of VAM colonization. For each sample, enough stems were pulled to collectively include at least 20 rootlets. The species counted was assumed to be Stipa comata due to the 2-3 year lag for germination typical of Oryzopsis hymenoides (SCS 1988). Random samples were chosen by walking to the center of the plot and selecting the closest grasses to the collector, in any direction, starting at the center and moving to the outside of the plot until sufficient roots were obtained. Small grasses were chosen to maximize success of pulling entire roots; soil compaction from construction and rainfall made long grass roots harder to obtain in entirety. Samples were not taken for VAM analysis from T17, the unwatered treatment; spring rainfall was above average (Needles District Visitor Center records, Canyonlands National Park) and irrigation had not yet been installed, so all treatment plots received equal water up to this point eliminating the

difference between T16 and T17. Subsamples were stored in 20 dram vials with 1:1 ethyl alcohol and water.

Samples were cleared and stained with Trypan Blue (Koske and Gemma 1989) within 1 wk of being pulled. Roots were heated in 2.5% KOH for 25 minutes at 90°C in a water bath. They were then rinsed with three changes of water: two with tap water and the final rinse with distilled water. Roots were acidified in 1% HCl for 1 hour. Staining the roots involved storage with acidic glycerol/Trypan blue for 20 minutes at 90°C in the water bath, rinsing with three water changes (two of tap water, one of distilled water), and destaining in acidic glycerol without Trypan blue where they remained until microscopic examination.

Roots were examined under a compound microscope and mycorrhizal colonization classed in 1 of 5 categories as follows:

Category	Colonization Rate (% root length colonized)
1	No Infection
2	>0 - 25%
3	>25 - 50%
4	>50 - 75%
5	>75 - 100%

VAM colonization was determined two ways; (a) definite colonization - percent root length occupied by aseptate hyphae in the presence of vesicles, and (b) probable colonization - percent root length occupied whether vesicles were present or not (Jim Trappe, personal communication). For definite colonization the colonization rate was recorded as category 1,

no colonization, in the absence of vesicles. Where vesicles were observed the colonization rate was recorded as estimated percent of root length occupied by hyphae. For probable colonization percent occupied by hyphae was the sole consideration for category placement.

Taxonomic identification of VAM fungi was accomplished by sieving spores from a soil sample and examining microscopically. The soil sample was collected as a conglomerate from five randomly chosen plots in the top 10 cm of soil during July of 1991. Spores were sieved using four screen sizes; sieving progressed through smaller screens in the order of 850 μm, 0.246 mm, 90 μm and 53 μm. No spores were found in the 53 μm sieve. Genera of mycorrhizal fungi were identified using keys by Schenck and Perez (1990).

Statistical Analyses

Data on VAM colonization and Stipa density was analyzed using multivariate analysis with Multiple Linear Regression tests. Mean densities of July and September Stipa measurements were log transformed to meet assumptions of normality and constance. Residuals of VAM measurements were distributed normally on an untransformed scale. The mean of each treatment was compared to the control of seed only plus water (T16) using an LSD multiple range test. Planned comparisons were made for treatments involving sugar and hay including each amendment in combination with other amendments

compared to the other amendments alone. Two analyses of VAM colonization were performed, one in which vesicles were required to indicate amount of VAM colonization greater than 0, and one in which aseptate hyphae were taken to indicate "probable" VAM (Jim Trappe, personal communication). Definite VAM colonization was regressed at July Stipa density to determine whether the treatments had the same pattern of effects and whether VAM colonization could be partially attributed to Stipa density. Definite VAM was then regressed at probable VAM colonization to determine whether similar factors determine hyphal colonization as vesicle formation. Both correlations were run with a Simple Linear Regression.

RESULTS

Stipa establishment

Stipa densities are reported for July and September measurements in Tables 2 and 3, respectively. None of the treatments produced significantly greater Stipa densities than the control treatment of seed only plus water (T16), while treatments with mulch resulted in significantly lower densities from both July and September measurements. Spring crust plus fertilizer (T13) had a significant negative effect in September and not in July. Likewise spring crust alone (T11) developed a fairly significant negative effect in September that was not demonstrated in July.

Several planned comparisons were made to identify effects of amendments not tested in isolation (Table 4). None of the comparisons for sugar or hay showed significant differences for July or September measurements.

The grass species on which density measurements were made could not be determined at this early stage of growth. Seed heads and more developed leaf structure are necessary to make positive species identification. It is probable, however, that Stipa comata was the species measured due to a 2-3 year delay of germination typical of Oryzopsis hymenoides (SCS 1988).

Vesicular-arbuscular mycorrhizae

Relative probable (aseptate hyphae) and definite VAM colonization (presence of vesicles) among treatments are reported in Tables 5 and 6, respectively. Six treatments increased definite VAM colonization by three to over five times above that of controls, and with a probability of 0.05 or greater: spring crusts alone (T11) and spring crusts with sugar added (T12), fall crusts with mulch added (T4) and with both mulch and sugar added (T6), mulch plus fertilizer (T9), and mulch plus sugar (T8). Of these, by far the strongest effect statistically ($p < 0.001$) was with spring crusts, spring crusts plus sugar, and fall crusts plus sugar and mulch. Spring crusts and spring crusts plus sugar did not differ from one another, indicating the effect was due solely to crusts.

Planned comparisons for effects of sugar and hay are listed in Table 7. Fall crusts plus sugar (T2) differed from fall crusts alone (T1) for definite and probable VAM, the sugar affecting definite VAM negatively and "probable" VAM positively. There was a difference between the combinations of fall crusts, mulch and sugar (T6) and fall crust plus mulch (T4), the sugar adding a synergistic positive effect. A difference also existed for probable VAM between sugar and mulch (T8) and mulch alone (T10), the sugar having a negative effect. There was no difference for the comparisons of spring

crust and sugar (T12) to spring crust alone (T11) nor for hay plus fall crusts (T7) to fall crusts alone.

The genus of VAM mycorrhizal fungi found in the soil and presumed to be associated with Stipa sp. was Acaulospora, forming endomycorrhizae with lobed vesicles (Schenck and Perez 1990). There was an insufficient number of spores to identify species, however the samples examined appeared related to A. longulata from the pale yellow coloring, the spore size in the 75 - 90 μm range, and the mucilaginous spore wall (Schenck and Perez 1990).

Definite VAM colonization and July Stipa density were highly uncorrelated from a Simple Linear Regression (r^2 = 0.01, p = 0.05). Definite and probable VAM colonization also did not show the same pattern of effects based on Simple Linear Regression (r^2 = 0.0625, p=.05).

Table 2. Mean July Stipa density for treatment groups. Treatments include water unless indicated otherwise. Significant differences were determined with the LSD multiple range test (* indicates difference from control (T16) at 0.05 level; LSD= 0.51 for 0.05 difference between two means).

Treatment	Mean No. Stipa stems
9 - mulch, fertilizer *	3.04
14 - s. crust, mulch *	3.10
8 - f. sugar, mulch *	3.43
5 - f. crust, fertilizer, mulch *	3.49
6 - f. crust, f. sugar, mulch *	4.30
4 - f. crust, mulch *	4.99
10 - mulch *	5.04
13 - s. crust, fertilizer	7.12
12 - s. crust, s. sugar	7.16
18 - s. crust, s. sugar, no water	7.73
7 - f. crust, hay	7.83
15 - fertilizer	8.47
11 - s. crust	8.58
3 - f. crust, fertilizer	9.76
16 - control: seed only	**9.84**
1 - f. crust	10.15
17 - seed, no water	11.22
2 - f. crust, sugar	11.34

Table 3. Mean September *Stipa* density for treatment groups. Treatments include water unless indicated otherwise. Significant differences were determined with the LSD multiple range test (* indicates difference from control (T16) at 0.05 level; LSD= 0.66 for 0.05 difference between two means).

Treatment	Mean No. *Stipa* stems
9 - mulch, fertilizer *	3.42
14 - s. crust, mulch *	3.56
8 - fall sugar, mulch *	3.58
10 - mulch *	4.86
5 - f. crust, fertilizer, mulch *	5.45
4 - f. crust, mulch *	5.78
6 - f. crust, f. sugar, mulch *	6.10
13 - s. crust, fertilizer *	9.42
11 - s. crust	9.34
3 - f. crust, fertilizer	11.19
15 - fertilizer	12.53
2 - f. crust, f. sugar	12.95
18 - s. crust, s. sugar, no water	14.22
12 - s. crust, s. sugar	15.49
1 - f. crust	16.68
7 - f. crust, hay	17.76
16 - control: seed only	**18.38**
17 - seed, no water	27.99

Table 4. Planned comparisons for July and September Stipa measurements.
X = significant difference, 0 = no significant difference (at 0.05 level).

For sugar effect:

July

Treatment a	compared to	Treatment b	Difference
2- f. crust, f. sugar		1 - f. crust	0
12- s. crust, s. sugar		11 - s. crust	0
6- f. crust, mulch, f. sugar		4 - f. crust, mulch	0
8- f. sugar, mulch		10 - mulch	0

September

Treatment a	compared to	Treatment b	Difference
For sugar effect:			
2- f. crust, f. sugar		1 - f. crust	0
12- s. crust, s. sugar		11 - s. crust	0
6- f. crust, mulch, f. sugar		4 - f. crust, mulch	0
8- f. sugar, mulch		10 - mulch	0

For hay effect:

July

Treatment a	compared to	Treatment b	Difference
7- f. crust, hay		1 - f. crust	0

September

Treatment a	compared to	Treatment b	Difference
7- f. crust, hay		1 - f. crust	0

Table 5. Mean definite colonization of VAM on Stipa. Treatments include water unless indicated otherwise. Significance was determined with the LSD multiple range test (* indicates difference from control (T16) at 0.05 level; LSD=14 for 0.05 difference between two means).

Treatment	Mean % definite VAM Colonization
2 - f. crust, f. sugar	6
16 - control: seed only	**10**
10 - mulch	11
15 - fertilizer	19
7 - f. crust, hay	20
18 - s. crust, s. sugar, no water	21
14 - s. crust, mulch	24
3 - f. crust, fertilizer	26
1 - f. crust	26
5 - f. crust, fertilizer, mulch	26
13 - s. crust, fertilizer	29
9 - mulch, fertilizer *	31
4 - f. crust, mulch *	31
8 - f. sugar, mulch *	37
12 - s. crust, s. sugar *	44
11 - s. crust *	47
6 - f. crust, f. sugar, mulch *	54

Table 6. Mean probable colonization of VAM on Stipa. Treatments include water unless indicated otherwise. Significance was determined with the LSD multiple range test (* indicates difference from control at 0.05 level; LSD=19 for 0.05 difference between two means).

Treatment	Mean % probable VAM Colonization
16 - control: seed only	35
15 - fertilizer *	51
14 - s. crust, mulch *	56
18 - s. crust, s. sugar, no water *	60
1 - f. crust *	61
3 - f. crust, fertilizer *	61
7 - f. crust, hay *	61
13 - s. crust, fertilizer *	62
9 - mulch, fertilizer *	64
8 - f. sugar, mulch *	65
5 - f. crust, fertilizer, mulch *	65
11 - s. crust *	68
10 - mulch *	68
4 - f. crust, mulch *	69
6 - f. crust, f. sugar, mulch *	74
12 - s. crust, s. sugar *	75
2 - f. crust, f. sugar *	78

Table 7. Planned comparisons for Definite and Probable VAM formation.
X = significant difference, 0 = no significant difference (at 0.05 level).

For sugar effect:

Definite

Treatment a	compared to	Treatment b	Difference
2- f. crust, f. sugar		1 - f. crust	X
12- s. crust, s. sugar		11 - s. crust	0
6- f. crust, mulch, f. sugar		4 - f. crust, mulch	X
8- f. sugar, mulch		10 - mulch	0

Probable

Treatment a	compared to	Treatment b	Difference
2- f. crust, f. sugar		1 - f. crust	X
12- s. crust, s. sugar		11 - s. crust	0
6- f. crust, mulch, f. sugar		4 - f. crust, mulch	0
8- f. sugar, mulch		10 - mulch	X

For hay effect:

Definite

Treatment a	compared to	Treatment b	Difference
7-f. crust, hay		1 - f. crust	0

Probable

Treatment a	compared to	Treatment b	Difference
7-f. crust, hay		1 - f. crust	0

Discussion

Stipa establishment

For both measurement periods, all treatments with mulch resulted in lower Stipa density than the control, whereas no other treatment differed from control. By September, most treatments with fertilizer also had significantly lower Stipa density than controls. Analysis of treatments is discussed with regard to each amendment below.

Straw mulch

The negative effect of mulch is in contrast with the literature and was a surprising result. Mulch has been found important in moderating moisture in semiarid systems (Wilson and Wilson 1992) for reasons including reduced moisture loss, lowered soil temperatures, reduced raindrop impact, and slower soil and water movement (McKell 1978). Other benefits of mulch have included retention of seed and fertilizer, inhibition of soil-borne pathogens (Wilson and Wilson 1992) and reduced wind velocity (Kay 1978, Hodder 1977). In this experiment the mulch may have created soil pathogen activity, causing damping off of seedlings. Soil temperatures and light may have also limited seedling establishment from excessive cover.

Competition from annual weeds imported with the mulch may deter its benefits (Gould et al. 1975), as may have happened in this experiment. The Hilaria seed heads of the mulch were purportedly removed, though Hilaria seedlings

germinated, which may have reduced Stipa density in treatment plots with mulch. The mulch may have created environmental conditions which were unfavorable to Stipa establishment for the first growing season. The organic matter and carbon added to the soil may be favorable for establishment of Stipa sp. in following years.

Cryptobiotic soil crusts

Adding topsoil has been favorable in semi-arid restoration in providing a soil micro-organism inoculum and native seed source (McKell 1978), re-establishing desirable plant species (Skujins and Allen 1986), increasing infiltration rates (Hodder 1978), and encouraging establishment of all plant cover (Cotts et al. 1991). Cryptobiotic crusts have been found to stabilize soil in arid systems (Campbell et al. 1989); the physical improvement and protection of arid soils may enable rehabilitation of arid lands (Isichei 1990). The singular cryptobiotic crust treatments (T1 and T11) in this study did not lead to increased Stipa density as predicted, though the effects of trampling may have impeded their effect. Fall crusts alone had no effect in either season while spring crusts had a negative effect at the 0.07 significance level.

Soil compaction and crust debility resulting from human travel over the soil from numerous measurements is a possible explanation for the failure of crusts to enhance Stipa establishment. All treatment plots were measured the

same number of times, so this effect applies to all treatments, though the predicted effect may have been reduced due to trampling. Past studies have recorded loss of total crust cover (Beymer et al. 1992) and cryptobiotic species diversity (Anderson et al. 1982) as a result of livestock grazing. Machinery compaction and tillage systems such as rotary and chisel plow have also reduced the rate of water infiltration in arid land agricultural fields (Abo-Abda and Hussain 1990).

Trampling appeared to compact the soil which may have limited infiltration and/or the water- and nutrient-retention capacity of the crusts. Stipa establishment may have subsequently been reduced due to small roots without the capacity to access nutrients and water (SCS 1988). However, neither fertilization nor watering improved Stipa establishment suggesting that nutrients and water were not limiting at this stage of the experiment. It is also possible that nutrients and water were not able to infiltrate down to the level of the roots, and may have evaporated on the surface before plants could utilize them.

Cryptobiotic crusts accumulate significant amounts of soluble carbon in fall and less so in spring, attributable to carbohydrates generated by the crust during photosynthesis (Beymer and Klopatek 1991). Various species of cyanobacteria have been associated with excretion of carbon, which have also been closely associated with

bacteria that assimilate the excreted material (Bauld and Brock 1974). Carbohydrate contents of crusts has been found to peak two days after the major spring thaw in Antarctica (Tearle 1987). Mosses have also exhibited carbohydrate leakage in a brief pulse for a few minutes following desiccation and rehydration (Beymer and Klopatek 1991). The contribution of carbon by crust organisms has been found nearly double in concentration in the fall as the spring (Beymer and Klopatek 1991). This may reflect carbon accumulation from photosynthesis over the summer.

Above-average spring rains would have been thought to increase available carbon to the soil from the crusts and encourage microbial activity. Soluble carbon has been found to move to lower levels in the soil within 10 days of photosynthesis (Beymer and Klopatek 1991). It seems logical to conclude that soluble carbon must thus be quickly utilized following availability or may be leached out of the plant-soil system.

Fertilizer

Desert soils are typically limited by nitrogen, phosphorus (Bauer et al. 1978) and potassium (Khudairi 1969) as well as characterized by low C-N ratios (Cundell 1977). Fertilizer was added here to supply limiting nutrients to the system with the expectation that the competing annual plants would use the nutrients to the detriment of perennial grasses.

Fertilization of plant communities occupying highly infertile sites has repeatedly been found ineffective for plant establishment and growth in infertile environments. Moderate nutrient additions (a 50% increase in annual nutrient flux) may be immobilized by inorganic adsorption and chemical solubility on infertile sites (Chapin III et al. 1986). This may also make a site infertile by tying up nutrients that seasonally become available. High fertilization has decreased the relative abundance of Stipa comata on a native range in Alberta (Johnston et al. 1967). In another study nitrogen increased production of Bromus tectorum, a weedy annual, and reduced production of perennial grasses in central Washington (Sneva 1963). A study in southeastern Oregon similarly found a 50% depression of a native grass, Agropyron spicatum, while Bromus tectorum increased by 600% over a 4-year period with repeated ammonium application (Kay 1966).

For my study, average Stipa density for the fertilizer only treatment (T15) was 30% lower than the control for the September measurement, however the difference was not statistically significant With more replications we might have demonstrated the hypothesized fertilizer effect.

Sugar

Disturbed grasslands are typically characterized by increased nitrifying activity (Rice and Pancholy 1973), encouraging annual weeds which can rapidly absorb available

nutrients (Costello 1944). This was expected to be countered with the addition of sugar; sucrose was added as a rapidly utilizable energy source for the purpose of increasing decomposer biomass, temporarily decreasing available soil nitrogen. Addition of sucrose as an amendment to semiarid systems in the past has had marginal to no success increasing the density of perennial grasses and forbs: Sugar had no impact on the growth of Artemisia tridentata in a Great Basin study (Miller et al. 1991), lowered the decomposition of litter in a semiarid prairie (Coleman et al. 1990), and only marginally increased the success of perennial grasses at a semiarid sagebrush site (McLendon and Redente 1991).

Stipa densities in this experiment similarly did not respond to added sugar. Planned comparisons to test the effect of sugar include fall crust plus sugar (T2) with fall crust (T1), spring crust plus sugar (T12) with spring crust (T11) and fall crust, sugar and mulch (T6) with fall crust plus mulch (T4). In none of these cased did added sugar result in a significant difference.

Hay

The hay mixed into the soil was intended to provide aeration and a source of carbon with decomposition. Hay with fall crusts (T7), the only treatment involving hay, did not produce a significant difference in Stipa density in

relation to the control treatment nor in relation to fall crusts alone (T1).

Water

Suprisingly, the highest average Stipa densities were in unwatered control treatments in September, although they did not differ significantly from the watered controls. Above-average rainfall occurred during months of March and May (11 cm between March and May versus the average 8.7 cm, Needles District Visitor Center records, Canyonlands National Park). The added water may have increased plant growth of all treatments and mitigated the comparison of watered and unwatered treatments.

Water is thought to be a major limiting factor to nutrient capture and plant productivity in the Great Basin (Skujins and Allen 1986, Allen and Allen 1984), and often determines the success or failure of reclamation efforts (Thames 1977). Irrigation has generally been recommended as a safeguard against drought in the early years of revegetation of arid lands (McKell 1978). Increasing soil moisture has facilitated nutrient uptake and plant growth in past studies (Ries and Day 1978), and increased the relative abundance of perennial grasses over S. kali in a dry grassland system (Lauenroth et al. 1978). Establishment of Stipa comata has been improved in past research with increased soil moisture (Costello 1944).

Analyses of field studies classically produce unexpected results due to a multitude of confounding factors, as did this study. Factors which may have influenced differences in Stipa density not accounted for by treatments include differences in substrate textures and depth, pre-existing micro-organisms, and the effect of rabbits clipping the stems, though there is no evidence these effects differed among treatments and the randomized block design should have accounted for differences. The length of this study may be insufficient to determine the eventual relative Stipa density or its role in community dynamics. Past studies of semiarid grassland restoration and rehabilitation have been measured for 2- to 3-year periods (Reeves et al. 1979, Allen and Knight 1984, Piemiesel 1951). Further monitoring of the study plots may provide more conclusive evidence of Stipa dynamics.

Vesicular-arbuscular mycorrhizae

VA mycorrhizae are thought beneficial in arid ecosystems for many reasons, including increasing water- and nutrient-absorption capacity, particularly phosphate-absorbing surfaces of perennial grass roots in low-phosphate soils (Allen 1991). VAM are believed to require a relatively continuous cover of host plants in order to persist in soils, and soil disturbance has been shown to significantly reduce VA inoculum potential (Allen

and Boosalis 1983). There was a poor correlation between Stipa density and mycorrhizal colonization. This is in contrast to what was predicted, in that extent of colonization is typically correlated with nutrient absorption of the plant and consequent growth (Harley and Smith 1983, Clapperton and Reid 1992).

All treatments resulted in greater probable VAM formation (presence of aseptate hyphae only). However, definite VAM (vesicles present), was significantly increased ($p = 0.05$) by only six treatments: spring crusts, either with or without sugar; fall crusts with mulch or with mulch and sugar; mulch with sugar; and mulch with fertilizer. Some treatments had quite different effects on probable and definite colonization. For example, fall crusts plus sugar did not influence definite colonization at all, but had a very strong effect on probable colonization. Mulch followed a similar pattern. Since vesicles are generally formed sometime after initial colonization (Harley and Smith 1983), it is reasonable to assume that the presence of vesicles in this study indicate colonization that occurred earlier in the growing season. Except for fertilizer, I will discuss treatment effects primarily in terms of carbon either as a source of energy and structure (cryptobiotic crusts, mulch, hay) or solely as an energy source (sugar). My discussion will focus on definite colonization.

Carbon as an energy source - Sugar

Sugar adds an immediate energy source which may have activated the micro-organisms. A number of comparisons were made to isolate the effects of sugar. Fall crusts plus sugar (T2) compared to fall crusts alone (T1) differed for definite and probable VAM. The effect of adding sugar to fall crusts was extremely negative for definite VAM and extremely positive for probable formation. Bacteria have been found to inoculate host plants simultaneously with mycorrhizal fungi. The presence of bacteria and microflora in the soil in general may enhance or suppress mycorrhizae and plant growth (Linderman 1987). Some types of bacteria require an external source of amino acids and/or vitamins secreted by plant roots in an undisturbed ecosystem (Richards 1987). Microflora also contribute to the production of exudates in the rhizosphere. Sugars have been identified as exudates, with glucose being one of the most abundant. The sugar added may have stimulated bacteria which encourage mycorrhizal formation, though later than formation without sugar. The positive effect on possible VAM would then reflect increased hyphal development and the negative definite VAM would be reflective of later initiation of formation not yet to the point of vesicle development.

A comparison of fall crust plus sugar and mulch (T6) to fall crust plus mulch (T4) differed for definite VAM.

Treatment T6 created a synergistic effect where the additive effects of fall crust plus sugar and fall crust plus mulch (6% + 31% = 37%) is less than the treatment with all three (54%). The synergism produced an extra 17% colonization that cannot be explained as additive effects of the treatments. The mulch provided a slower, more continuous energy supply, some nutrients from decomposing Hilaria, as well as vitamins, amino acids, or other organic acids, normally provided by plant secretions or decomposition (Richards 1987). The sugar provided a quick energy source which may have stimulated bacteria that encourage formation. The mulch may have also provided aeration and water-holding capacity.

Spring crust plus sugar (T12) compared with spring crust alone (T11) did not differ for definite or probable VAM, indicating the spring crusts were responsible for the positive effect. Sugar plus mulch (T8) compared to mulch alone (T10) differed for probable but not definite VAM. Sugar probably stimulated VAM-enhancing bacteria, though probably the formation developed later. Though the mechanism is not directly discernible, it can be inferred that sugar can be expected to lead to increased VAM colonization.

Carbon as energy and structure

Desert ecosystems are characterized in part by low organic matter (Cundell 1977), the retention of which is

critical to preserve nutrients and reduce erosion (Paul 1976). The presence of organic matter is thought key to developing structure in sandy soils, while the amount and constituents of the pool of organic matter is vital to the functioning of an ecosystem (Forster 1990). Treatments with straw, hay, and cryptobiotic crusts potentially provided structure to physically hold together the soil particles, and carbon as utilizable energy as the filamental structures broke down.

High organic matter usually promotes VAM establishment and persistence (St. John and Coleman 1983, Harinikumar et al. 1990), though when carbon is a major limiting factor the fungus has been found to act more as a pathogen than mutualist (Behlenfalvay et al. 1982). Many micro-organisms increase soil organic matter. Cyanobacteria, a major component of the cryptobiotic crusts, fix atmospheric nitrogen which stimulates the growth of micro-organisms. The diversity of a microbial population is site-specific, reflective of the organic matter content (Alexander 1965). Utilization of cyanobacteria and other nitrogen-fixing organisms of the soil biota has been recommended for improved arid land revegetation though the manipulation of soil biota has not been greatly undertaken (El-Tayeb and Skujins 1989).

Mulch

The soil ecosystem is thought to greatly benefit from any organic mulch, leading to stimulated mycorrhizal fungi and a root system protected from deleterious infection (Levisohn 1956). VA fungi have also colonized more heavily with the greater aeration and breakdown products of bacteria and other micro-organisms mulch provides (Wilson and Wilson 1992). Mulch alone did not increase definite colonization, nor in combination with spring crust, or fall crusts and fertilizer. Though mulching did not improve mycorrhizal formation, environmental conditions created by the mulch may have been beneficial to the overall ecosystem. These conditions include temperature reduction from shading, a physical impediment to erosion, and protection of the root system from infection (Levisohn 1952).

Hay

Hay plus fall crusts (T7) did not differ from fall crusts (T1) for definite or probable colonization.

Cryptobiotic soil crusts

Spring crusts had a strong positive effect on VAM, while fall crusts did not have an effect unless amended with sugar and/or mulch. I discuss crusts in relation to VAM, followed by possible differences of spring versus fall crusts.

Mycorrhizal inoculum may have been imported with the crusts. The inoculum potential has rarely been retained as

a result of topsoil retention when stockpiling interceded removal and replacement (Miller 1987). Long fallow periods have been found to reduce levels of mycorrhizal colonization and growth of Helianthus anuus (Thompson 1987). The soil crusts should have also improved water retention and reduced erosion forming a protective blanket for the soil ecosystem (Anderson et al. 1982, (1)). Spores could have then been trapped by the crusts and been in closer contact with the Stipa increasing inoculum potential. Immediate re-application should have saved the micro-organisms from desiccation (McKell 1978) for the spring crusts in this experiment.

Organic volatiles serve as the sole energy and carbon source for some microbes (Stotzky and Schenck 1976). Numerous organic volatile substances have inhibitory and stimulative effects on both microbial activity and plant growth. The volatiles are produced by biotic and abiotic sources. Biotic producers include soil microbes, seeds, living and dead plant matter, and animals, while the major abiotic sources include combustion of fossil fuels, industrial processes, burning and pesticides. Fungi produce a great diversity of volatiles (Stotzky and Schenck 1976), though the end effect is difficult to determine due to a great number of organisms and factors which may have an influence.

The effect of volatiles on mycorrhizal colonization cannot be determined without collective information about all volatiles affecting this system. The interactions of microbes in the rhizosphere are selective for each set of organisms (Azcon et al 1989). Ethylene has been found to form in soils as a result of microbial activity, encourage fungistasis in soil and adversely affect plant growth (Lindberg et al. 1979), though other volatiles are likely influential on growth dynamics as well. The great diversity of possible effects of volatiles further illustrates the complexity of the ecosystem.

Greater colonization of spring versus fall crusts may have to do with increased biological activity of the soil biota in the spring (Beymer and Klopatek 1991). Organisms in the fall crusts probably became less active shortly after application. The fall crusts effectively were "stockpiled" over the winter resulting in reduced inoculum potential and colonization.

Spring is the season of maximum precipitation in the Great Basin desert (Loope 1978), which in combination with increasing day length and warmer temperatures triggers biological activity and micro-organisms in the soil environment become active. The simple carbohydrates and amino acids given off by cryptogamic plants would stimulate microbial activity. Rhizosphere bacteria likely to stimulate VAM formation are also more common in the spring

(Richards 1987). The resulting crusts the following spring may not contain as many viable organisms as the more recently transferred spring crusts.

The addition of organic matter to the system under study may have affected plant establishment and mycorrhizal colonization by providing limiting nutrients, vitamins, amino and/or organic acids, stimulating mycorrhizal colonization, and/or instigating production of volatile substances which may be either inhibitory, stimulative or have no effect on both plant growth and fungal activity.

Fertilizer

The response of VAM formation to the nutrient environment has been found to be curvilinear, in which formation increases with stressful environmental conditions to a point, and then declines (Allen 1991). At the low end of the curve there may be fewer or less nutrient-rich plants to support formation, and less inoculum potential. The nutrient increase would support more colonization until the plant cover is dense enough that resource allocation to the fungi is no longer biologically worthy with mounting competition from other plants.

Fertilizer alone did not result in greater colonization. The lack of response for fertilizer further supports the curvilinear relationship where the nutrient level of the soil may have been near the point of maximal formation before the fertilization. The nutrients added may

have not been assimilable to the plants before leaching from the upper soil layers (Beymer and Klopatek 1991). Even if in the soil long enough for absorption, the addition of fertilizer also may not have resulted in more nutrient-rich plants; there is evidence that plants accustomed to nutrient-poor environments do not assimilate artificial fertilization (Chapin III et al. 1986).

The inhibition of mycorrhizal colonization from inorganic fertilization concurs with results of past studies. High rates (Skujins and Allen 1986) and intensive use of inorganic fertilizers have drastically inhibited VAM formation (Haymen 1982). Fertilization has resulted in decreased root colonization of Andropogon gerardii, an arid-land grass (Hetrick et al. 1988), as well as decreased microbial biomass, soluble organic carbon, and soil organic matter (Klein 1989).

Water

Above average spring rainfall partially reduces the extent of water exclusion, as with Stipa density. The control treatment for water with seed only and no water (T17) was not measured for definite or probable VAM colonization. By the month of June when VAM measurements were taken, spring rainfall had been great enough to not allow for a valid comparison. This was not true for the Stipa measurements when drying out of the non-water treatments had been allowed to occur. The one treatment

without water measured for VAM analysis (T18) resulted in a marginal increase above the control compared to the other treatments. Results are hypothesized for years of average rainfall.

A significant reduction in mycorrhizal colonization would be expected without irrigation based on past research, though irrigation in arid ecosystems can also induce salinity and inhibit establishment of VAM (Hirrel 1981). Another possibility for a year with average rainfall is a waterlogging effect which may reduce colonization from excessive water. The numbers of hyphal entry points on roots hairs have been found to be lower in wet soil, especially in dry habitats (Harley and Smith 1983). Desiccation of mycorrhizal fungi is common in arid ecosystems, requiring resistant propagules to survive drought (Gray and Williams 1971). Fungal colonization may be less affected by lack of water than Stipa density as less moisture has been found necessary for spores than plant seeds to germinate (Johnson 1977).

Revegetation

The measurements taken in this experiment are two assessments of ecosystem health, implicating above- and below-ground functions. The volume and diversity of organisms and relationships which act on this system make the possibility of knowing and understanding all of them

unlikely. Identifying organisms, functions and processes critical to maintaining the physiological processes which make up the ecosystem may provide the initiative needed to rehabilitate and/or restore the vast quantity of disturbed arid lands.

While identification of deterministic ecological functions spans a range of elements, actors and processes which may influence each other, as in a food web, improvement of an ecological system may require a total systems approach encompassing as much information about deterministic parts of the ecosystem as is feasible. In that naming and understanding the function of every member of the soil biota is neither possible nor likely useful to replication, protection of a system must assume every organism is essential and protect the land in entirety. Determination of which organisms have a synergistic effect on reproduction and persistence of the ecosystem is the issue for rehabilitation of land already denuded.

Techniques involving manipulation of soil biota have been recommended for arid land rehabilitation. Included are utilization of available organic matter, cyanobacteria and other N-fixing members of the soil biota, and mycorrhizae for improved revegetation efforts (El-Tayeb and Skujins 1989). From this study the addition of sugar, mulch and soil crusts provided a favorable environment for mycorrhizal colonization, while fertilizer did not add significantly to

formation. Fertilization is not recommended for disturbed areas where nutrients are limiting and competition by annuals is probable, while the use of renewable organic amendments is encouraged.

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MICROBIAL ANALYSIS OF REVEGETATION PLOTS IN CANYONLANDS NATIONAL PARK
PART II: TOTAL AND ACTIVE BACTERIA AND FUNGI

Bacterial/Fungal Analysis of Revegetation Plots in Canyonlands National Park

Due to cost constraints, only a subset of treatments were analyzed for total and active bacterial and fungal biomass. Treatments with the highest and lowest numbers of Stipa (Oryzopsis) hymenoides were included, along with at least one example of each type of treatment. Experimental plots were divided into two types of plots, based on the rockiness of the soils, and treatments were chosen from each. Final treatments selected included the following:

Rocky plot treatments:
- 4: Seed only (SD)
- 5: Mulch (M)
- 6. Sugar (S)
- 18. Undisturbed control

Non-rocky plot treatments:
- 1: Fertilizer (F)
- 7: Fall crust/ Hay mixed in (FC/H)
- 8: Mulch and sugar (M/S)
- 12: Spring crust and sugar (SC/S)
- 14: Spring crust and mulch (SC/M)
- 15: Fertilizer
- 17: Seed only, no water
- 18: Undisturbed control

All plots received supplemental water to imitate average rainfall except for non-rocky treatment 17.

RESULTS

Tables 1-6 present the results of bacteria and fungal biomass analyses. Total Stipa numbers, as well as Salsola cover and biomass, are included for comparative purposes. Although results represent 5 samples from 7 replicates of each treatment, variability was still very high. This made demonstrating statistical differences difficult, and in several cases, more replicates were clearly needed. Where this was the case, the differences are still discussed, but referred to as trends.

Overall, disturbance and/or additional water resulted in increases in both active fungal and bacterial biomass, as all treatments tended to be higher than the undisturbed control. Sugar treated plots showed a statistically significant 3.5-5.5-fold increase of active fungal biomass when compared to the control for both rocky and non-rocky plots. Active bacterial biomass did not show statistical differences, but again all treatments tended to be higher than the control.

Total microbial biomass did not show such a pattern. Total fungal biomass was higher in rocky treatments than the control, while non-rocky treatments were not different. Within non-rocky treatments, those plots with organic matter added tended to increase fungal biomass, as would be expected.

Total bacterial biomass showed some statistical differences. Sugar treatments in both the rocky and non-rocky plots showed 2-fold increases over the control. In rocky plots, a 2-fold increase was seen over the seed and mulch treatments; in the non-rocky plots, increases were seen over the fertilizer and seed only treatments.

The ratio of active to total fungal biomass was significantly increased by the sugar treatment in both rocky and non-rocky treatments when compared to the control. In non-rocky plots, this ratio in sugar treatments was significantly greater than all the other treatments as well. No significant differences were seen in any of the other ratios, including active/total bacteria, active fungi/bacteria or total fungi/bacteria. However, there were some interesting patterns. Sugar treatments showed higher active fungi/bacteria ratios than other treatments, and lower total fungi/bacteria ratios.

Correlation analysis shows that total fungi/bacteria ratio is negatively correlated with Stipa numbers at the 0.06 level, with r = 0.27. This was the only predictive variable found. Multiple regression showed that adding active fungi to the equation increased the r value to 0.40, but active fungi was not statistically significant.

DISCUSSION

Past work has demonstrated that bacteria tend to dominate in alkaline soils, in systems with low organic matter and in systems where organic matter is mixed into the soil profile. Systems with a heavy litter layer, where soils are susceptible to drying, or where soils are acidic tend to be fungal-dominated. Therefore, one would expect undisturbed semi-arid grasslands to be heavily bacterially-dominated, since soils are alkaline, organic matter is low, and litter layers sparse. Since these soils are exposed to repeated drying, there is some favoring of fungal populations. Mixing soil layers, as is often the case in revegetation projects, should favor bacterial components even more, as litter is distributed throughout the soil. Greater bacterial dominance probably also leads to faster decomposition rates in deserts, as bacteria are more adapted than fungi to breaking down the less resistant litter found in these systems. In addition, fungal/bacteria ratios may influence the types of plants found in ecosystems, as faster decomposition rates lead to greater nutrient availability; therefore, this may favor annual plants.

Overall increases in microbial biomass can also result in greater

retention of nitrogen in desert systems, since the nitrogen is tied up in microbial biomass and not easily leached or volatilized. With the presence of microbial predators in these systems such as nematodes, spring nutrient flushes also can result when predator populations increase with warmth and moisture.

As predicted by previous work, disturbance to the grasslands in Canyonlands had no effect on total fungal biomass when treatments were compared to the undisturbed control. For treatments not using sugar, there was a small rise in total bacterial biomass which seemed attributable to disturbance alone, again as would be predicted by previous studies. Interestingly, there was little correlation between Stipa success and any measure of the microbial community. The total fungal/bacteria biomass ratio was the only factor correlated, and only weakly. This negative correlation, however, did indicate this grass tends to prefer a more bacterially-dominated system, as previous grassland work has shown.

Sugar had two effects on this system. First, it stimulated activity in both the bacteria and the fungi. Secondly, it lowered the fungal/bacterial ratio, clearly favoring bacteria over fungi. Results indicate that this ratio was well below that found in the undisturbed area.

As predicted, addition of organic matter (through surface mulch or hay mixed into the surface) increased the fungal/bacteria ratio relative to the other treatments. Interestingly, it more closely approximated the ratio found in the undisturbed system. This could indicate that the undisturbed system contained more resistant organic matter than found in the replaced soil of the treatments, or that the added organic material more closely resembled the naturally occurring litter layer. Both of these situations would favor fungi over bacteria. Sugar clearly swamped this effect, as seen in mulch/sugar treatment.

It is not known if the undisturbed control actually represents the most optimal germination and establishment conditions for grass seedlings. There may be microbial balances that are more favorable for seedling success than those found in established plant communities. Looking at the levels of bacteria and fungi in treatments with the most Stipa establishment, several differences from the control treatment stand out: successful Stipa plots differed from the undisturbed control in having higher active fungal biomass, lower total fungal biomass, higher active bacterial biomass, much higher total bacterial biomass, and much lower total fungal/bacterial biomass. Microbial biomass, both total and active, was higher as well. This would indicate that systems with greater microbial activity, as well as more bacterially dominated systems, than found in the undisturbed control, favor establishment of Stipa grasslands. Under these circumstances, sugar should enhance Stipa success, since it stimulates overall active biomass, as well as favoring bacteria over fungal populations. Sugar was also found to decrease the biomass of the competing exotic Salsola.

Further analysis of this data will prove valuable, as many relationships have yet to be examined. Understanding of the relationship of above-ground communities to below-ground food webs will enable us to better accomplish revegetation of arid land ecosystems.

	SALSOLA BIOMASS	SALSOLA COVER	STIPA	ACTIVE FUNGI	TOTAL FUNGI	ACTIVE BACTERIA	TOTAL BACTERIA
UNDISTURBED				0.38a	11.3	1.4a	6.0a
SEED	32	26	19.4a	0.33a	7.0	1.6a	8.7
MULCH	14	20	7.3b	0.64a	8.2	2.4b	6.2a
SUGAR	25	19	26.6a	1.4b	6.0	2.3b	14.5b
p	NS	NS	.05	.04	.23	.04	.03

Table 1. Rocky Plots: Effects of seed, mulch and sugar soil treatments on Salsola, Stipa, fungi and bacteria in rocky soils. Figures marked "a" and "b" designate statistical differences ($p < 0.05$).

	SALSOLA BIOMASS	SALSOLA COVER	STIPA	ACTIVE FUNGI	TOTAL FUNGI	ACTIVE BACTERIA	TOTAL BACTERIA
UNDISTURBED				0.38a	11.3	1.4	6.0a
FERTILIZER	52	33	13b	0.57a	9.4	2.1	8.2a
SEED	45	24	28a	0.69a	8.5	1.9	11.6a
FALL CRUST	54	36	17	0.89	9.2	2.6	8.8
FALL CRUST/ HAY	31	24	19a	0.95	13.8	2.5	7.9
SPRING CRUST/ MULCH	77	35	4b	1.0	11.4	1.7	9.7
MULCH/ SUGAR	23	24	3b	1.9b	12.8	2.6	12.3c
SUGAR/ SPRING CRUST	35	27	18a	2.1b	9.7	2.3	14.0b
p	NS	NS	.01	.04	.39	.23	.06

Table 2. Non-rocky Plots: Effects of various soil treatments on Stipa, Salsola, fungi and bacteria in non-rocky soils. Figures marked "a" and "b" designate statistical differences ($p < 0.05$). Figures marked "c" differ only from control plots.

	SALSOLA BIOMASS	SALSOLA COVER	STIPA	ACTIVE/ TOTAL FUNGI	ACTIVE/ TOTAL BACTERIA	ACTIVE FUNGI/ ACTIVE BACTERIA	TOTAL FUNGI/ TOTAL BACTERIA
UNDISTURBED	0			.04a	.24a	.27a	1.94
SEED	32	26	19.4a	.11a	.21a	.19a	.95
MULCH	14	20	7.3b	.17	.52b	.30a	2.22
SUGAR	25	19	26.6a	.32b	.32	.59b	.92
p	NS	NS	.05	.01	.05	.03	.32

Table 3. Rocky Plots: Effects of seed, mulch and sugar soil treatments on Salsola, Stipa and fungi and bacteria ratios in rocky soils. Figures marked "a" and "b" designate statistical differences ($p < 0.05$).

	SALSOLA BIOMASS	SALSOLA COVER	STIPA	ACTIVE/ TOTAL FUNGI	ACTIVE/ TOTAL BACTERIA	ACTIVE FUNGI/ ACTIVE BACTERIA	TOTAL FUNGI/ TOTAL BACTERIA
UNDISTURBED				.04a	.24	.27a	1.94
FERTILIZER	52	33	13b	.08a	.29	.29a	1.45
SEED	45	24	28a	.10a	.19	.41	1.01
FALL CRUST	54	36	17	.13a	.38	.34a	1.21
FALL CRUST/HAY	31	24	19a	.10a	.41	.44	1.86
SPRING CRUST/ MULCH	77	35	4b	.08a	.28	.65	2.09
MULCH/SUGAR	23	24	3b	.15c	.24	.68	.94
SUGAR/ SPRING CRUST	35	27	18a	.24b	.27	.77b	.88
p	NS	NS	.01	.01	.43	.05	.14

Table 4. Non-rocky Plots: Effects of various soil treatments on Salsola, Stipa and fungi and bacteria ratios in non-rocky soils. Figures marked "a" and "b" designate statistical differences ($p < 0.05$). Figures marked "c" differ only from control plots.

	ACTIVE FUNGI	TOTAL FUNGI	ACTIVE BACT.	TOTAL BACT.	ACTIVE /TOTAL FUNGI	ACTIVE /TOTAL BACT.	TOTAL FUNGI/ TOTAL BACT.	ACTIVE FUNGI/ ACTIVE BACT.	STIPA	SAIB BIO- MASS	SAIB COVER	TOTAL FUNGI + BACT.	ACTIVE FUNGI + BACT.
UN- DIST.	.38a	11.3	1.4a	6.0a	.04a	.24a	1.94	.27a				17.0	1.8
SEED	.33a	7.0	1.6a	8.7	.11a	.21a	.95	.19a	19.4a	32	26	15.7	1.9
MULCH	.64a	8.2	2.4b	6.2a	.17	.52b	2.22	.30a	7.3b	14	20	14.4	3.1
SUGAR	1.4b	6.0	2.3b	14.5b	.32b	.32	.92	.59b	26.6a	25	19	20.5	3.7
p	.04	.23	.04	.03	.01	.05	.32	.03	.05	NS	NS		

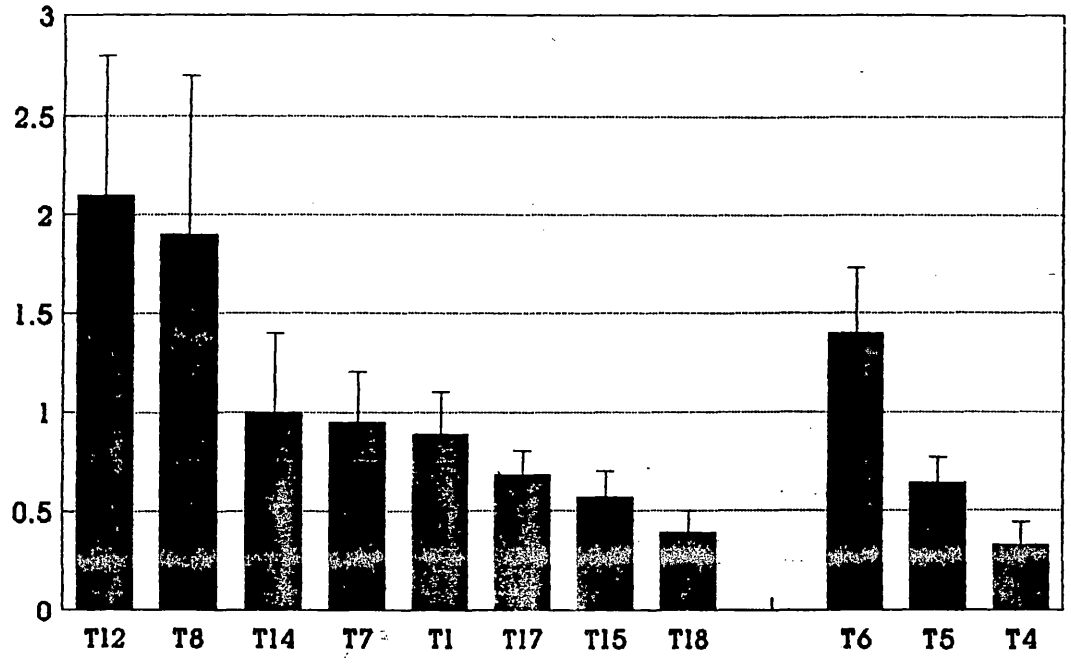
Table 5. Rocky Plots: Effects of seed, mulch and sugar soil treatments on Salsola, Stipa, fungi, bacteria and corresponding ratios in rocky soils. Figures marked "a" and "b" designate statistical differences ($p < 0.05$).

	ACTIVE FUNGI	TOTAL FUNGI	ACTIVE BACT.	TOTAL BACT.	ACTIVE /TOTAL FUNGI	ACTIVE /TOTAL BACT.	TOTAL FUNGI/ TOTAL BACT.	ACTIVE FUNGI/ ACTIVE BACT.	STIPA	SAIB BIO- MASS	SAIB COVER	TOTAL FUNGI + BACT.	ACTIVE FUNGI + BACT.
UN- DIST.	.38a	11.3	1.4	6.0a	.04a	.24	1.94	.27a				17.0	1.8
FERT.	.57a	9.4	2.1	8.2a	.08a	.29	1.45	.29a	13b	52	33	17.2	2.7
SEED	.69a	8.5	1.9	11.6a	.10a	.19	1.01	.41	28a	45	24	20.1	2.6
FALL CRUST	.89	9.2	2.6	8.8	.13a	.38	1.21	.34a	17	54	36	18.0	3.5
FALL CRUST / HAY	.95	13.8	2.5	7.9	.10a	.41	1.86	.44	19a	31	24	21.7	3.4
SPRNG CRUST / MULCH	1.0	11.4	1.7	9.7	.08a	.28	2.09	.65	4b	77	35	21.1	2.7
MULCH / SUGAR	1.9b	12.8	2.6	12.3c	.15c	.24	.94	.68	3b	23	24	25.1	4.5
SUGAR / SPRNG CRUST	2.1b	9.7	2.3	14.0b	.24b	.27	.88	.77b	18a	35	27	23.0	4.4
p	.04	.39	.23	.06	.01	.43	.14	.05	.01	NS	NS		

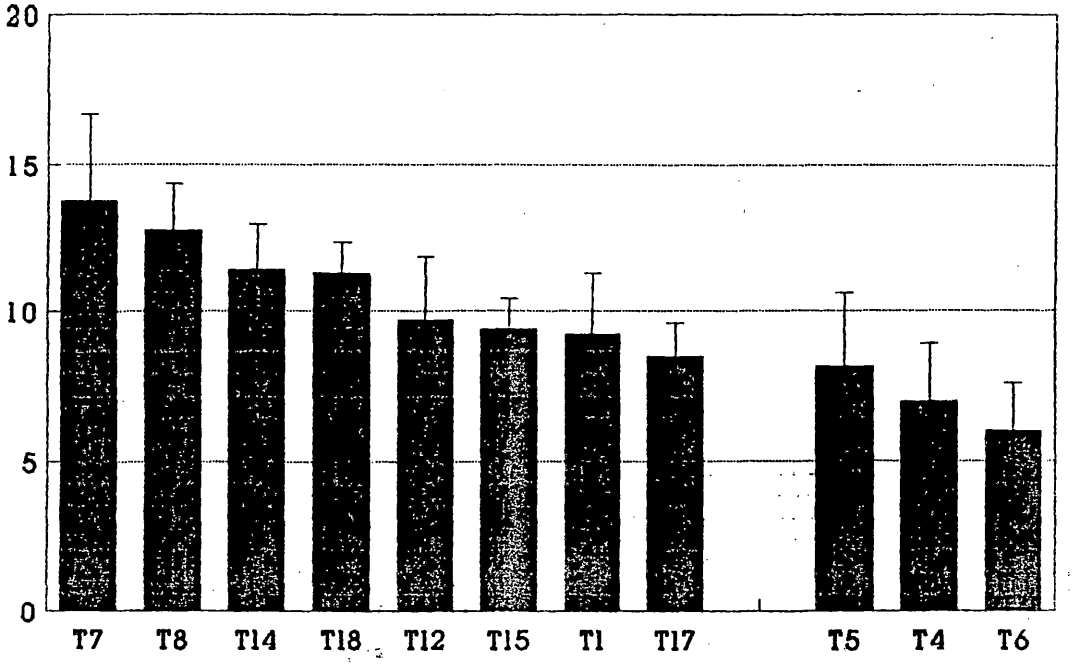
Table 6. Non-rocky Plots. Effects of various treatments on Salsola, Stipa, fungi, bacteria and corresponding ratios in non-rocky soils. Figures marked "a" and "b" designate statistical differences ($p < 0.05$). Figures marked "c" differ only from control plots.

Charts 1-4 are graphic representations of Tables 1-4. For statistical differences, see Tables 1-4.

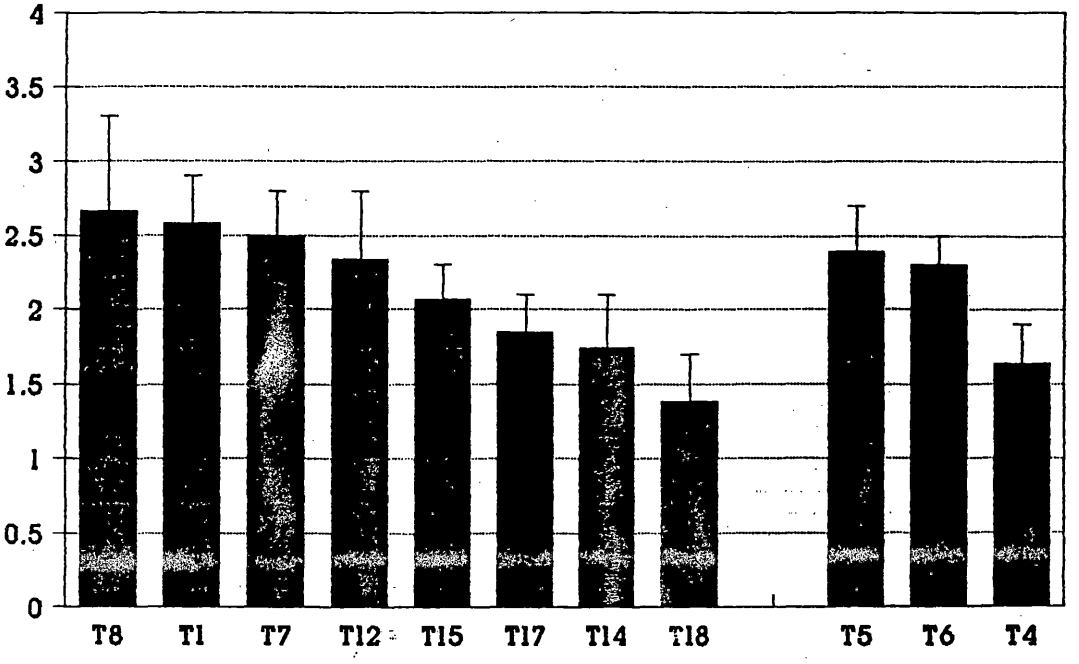
ACTIVE FUNGI NEEDLES 93



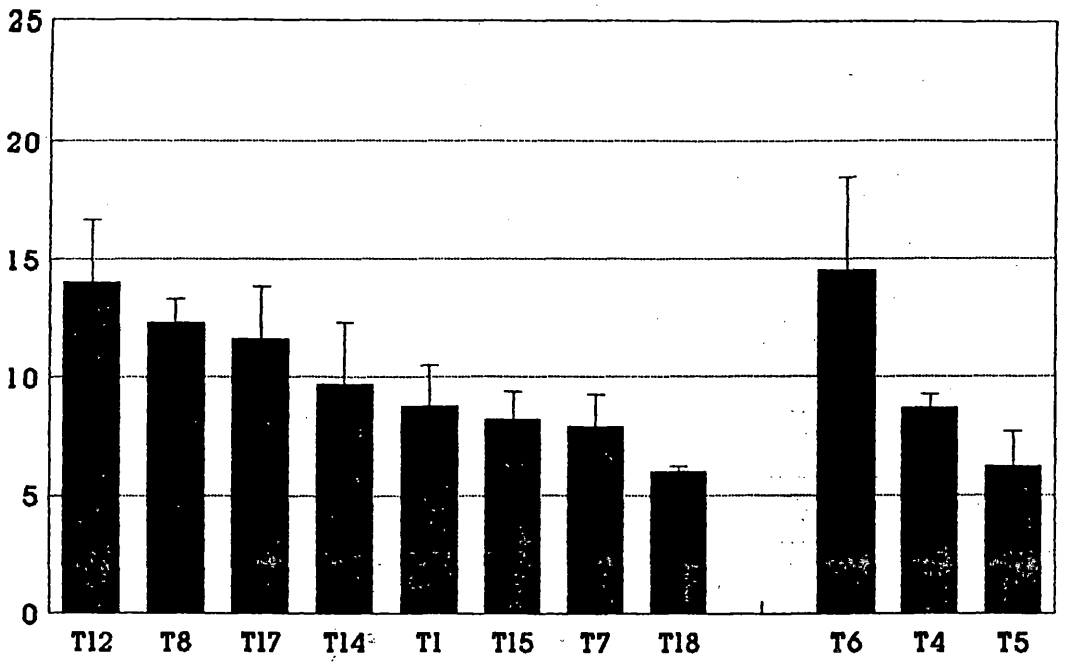
TOTAL FUNGUS



ACTIVE BACTERIA



TOTAL BACTERIA



PROPAGATION OF CRYPTOGAMS FOR USE IN LAND STABILIZATION REPORT

Prepared by

Jeffrey R. Johansen and Larry L. St. Clair
Principal Investigators

We proposed to isolate cyanobacteria from desert soils, propagate them in the laboratory, and then inoculate disturbed soils with a cyanobacterial amendment. We also proposed to study the inoculated plots to determine the degree of establishment of a crust, as well as the degree of stabilization of the soil surface. Dugway Proving Grounds was chosen as the site for field studies. Laboratory studies were to be conducted both at Brigham Young University and John Carroll University, under the supervision of Larry St. Clair and Jeff Johansen, respectively. This report will discuss the progress made thus far on the original proposal and its renewal.

RESEARCH OBJECTIVES:

1. Select and isolate one or more species of soil stabilizing cyanobacteria from the soil crust communities at Dugway Proving Grounds including Microcoleus vaginatus.
2. Evaluate the reproductive biology and ecology of those species of cyanobacteria selected for this project.
3. Develop a suitable growth medium for cyanobacterial isolates.
4. Develop a small-scale growth apparatus for culturing cyanobacterial isolates.
5. Propagate sufficient amounts of inoculum for field testing.
6. Evaluate alternative methods for field inoculation.
7. Inoculate field plots and monitor recovery of soil crust communities.
8. Quantify cyanobacteria on trampled, burned and undisturbed plots at Dugway Proving Grounds using epifluorescent microscopy. These data will be used to monitor the effectiveness of inoculation procedures as well as recovery patterns following fire and trampling disturbances.
9. Quantify and characterize eukaryotic algae from field plots using standard dilution plate techniques. This information will help to clarify interactions and relationships between cyanobacteria and eukaryotic algae in soil crust communities.
10. Perform sedimentation studies at field plots to determine if soil surfaces have been effectively stabilized following application of pelletized cyanobacterial inoculum.
11. Analyze soil chemistry at field plots in order to effectively evaluate the effects of pelletized cyanobacterial inoculum on soil chemistry.

INITIAL STUDIES

Soil samples were collected from Dugway Proving Grounds both in the sand dune area above our study site, and from the greasewood bottom near the SERL site in April of 1992. Cyanobacteria were isolated from these soils, and three species were chosen for this project: Microcoleus vaginatus, Nostoc punctiforme, and taxon originally identified as Scytonema hofmannii. The Scytonema species may belong be S. hofmannii, S. ocellatum, or even a Tolypothrix species. It has variable morphology in culture, and we plan to inoculate the algae on wet soil to determine what its most natural morphology is before publishing results of this study. In this report it will consistently be referred to as S. hofmannii. The Microcoleus vaginatus also changes its morphology when cultured in liquid media, taking on the appearance of an Oscillatoria. However, we are positive that our isolate is M. vaginatus because of its characteristic morphology at the time of isolation.

Growth in three media (Bold's Basal Medium, Z8, ASM-1) at two temperatures (25°C, 30°C) were tested. Growth of all three strains was best in Z8 media at 25°C. The strains were grown under continuous illumination at 50-100 $uE \cdot cm^{-2} \cdot s^{-}$

[1]. All three organisms grew best when started out in dim light in small containers. Nostoc and Scytonema grew better than Microcoleus, and we were able to inoculate Bellco 3 liter, aerated stir flasks with the former two species. Eventually, we learned that the growth of Microcoleus could be greatly enhanced by early aeration, and we now have that organism growing in the 3 liter flasks as well.

We have tried to use sterile technique as much as possible. However, since the cultures are not axenic (they have bacterial contaminants), rigorous sterile technique was not deemed necessary. Primarily, we have been careful to avoid green algal or cyanobacterial contamination. In order to do this, flasks are sterilized, and aeration is with filtered air (0.45 um pore size). Contamination has not been a serious problem, although it has occurred in some cultures. These have been replaced with inocula from uncontaminated cultures.

ENCAPSULATION

We have developed a protocol for encapsulation of the cyanobacteria. Cyanobacteria are encapsulated in 1.0% alginate (Kelgin MV). This requires 10 g alginate/liter liquid. Alginate is difficult to get into culture. The alginate will form insoluble aggregates unless high shear mixing is used. At first we used a drill with paint stirrer attached. Later we found that a kitchen blender worked well and was less awkward to use. Once in solution, which has the consistency of soft gelatin, the alginate is put into a funnel so that it will drip slowly into a 2% calcium chloride solution, which causes the alginate to set into firm, small pellets. We originally tried 1.0%, 1.5%, and 2% alginate in 2%, 3.5%, and 5% calcium chloride. The lower concentrations gave good results and were adopted for the remainder of the study.

Harvesting the algae was a slow process. We had 1-liter funnels which would drip into 2 liter flasks containing a liter of 2% calcium chloride solution. After setting up (alginate needs at least 1 hour in the solution), the alginate pellets were transferred to unsterile media. This allowed the excess calcium chloride to diffuse out of the beads. If not removed, it was postulated that the calcium chloride could be deleterious to the cyanobacteria and would contribute to the conductivity of the soil, both undesirable outcomes. Unsterile Z8 media was used to avoid osmotic shock and to provide some nutrients for the cyanobacteria, thus enhancing the chances of vigorous growth when the cyanobacteria were inoculated on the soil. Generally, the pellets were allowed to sit in unsterile media over night, although 4 hours was sufficient time for the calcium chloride to diffuse out of the pellets.

After sitting in the unsterile Z8, pellets were removed from the media by pouring the liquid through coarse mesh screens. The pellets were then placed on trays to dry, which usually took 1-3 days. After drying the pellets were kept refrigerated.

We wished the cyanobacterial amendment to have a uniform amount of cyanobacteria. This was done by weighing the amount of cyanobacteria that went into the solution. We decided on using 2.5 g algae/liter. Cultures were typically harvested when they had about 5 g algae/liter, so they usually had to be diluted before the alginate was added.

FIELD STUDIES

A total of 18 permanent blocks (5X9m) in an area 30X30m near the SERL site in Dugway Proving Grounds were delineated. In each block, six permanent meter square plots were established (Fig. 1). Two of the plots were burned, two were trampled, and two were left undisturbed. Treatments were assigned randomly to adjacent pairs of plots. For each pair of plots, one plot was designated to be eventually inoculated, with the other designated as an uninoculated control. Half the blocks were designated to be used to enumerate cyanobacteria by chlorophyll a and microscopic analyses, while the others were

designated sedimentation blocks. All assignments or treatments, etc. were determined randomly before going to the field to set up the plots (Fig. 1). By using blocks and plots set up systematically, and assigning treatments randomly, we felt we could obtain a representative and unbiased sample suitable for parametric statistical analyses later on.

Burning of designated quadrats was accomplished by placing a steel barrel (1m diameter) over the plot and introducing propane flame jets from holes near the bottom of the drum for 20 seconds. This produced an evenly burned surface without risk of starting rangefires. The method has been studied and found to mimic the temperatures and duration experienced in a rangefire. Plots were burned for 15 seconds at temperatures of 300-900°C. Relative humidity at the time of the burn was 19.5%. The burn was conducted in the fall of 1992.

Trampling was conducted by walking repeatedly on designated plots. Trampling was done twice in the fall of 1992. The crust was thoroughly disrupted by the trampling treatment.

Surface soil samples for soil analysis were taken outside of the plots to avoid destruction of crust communities within the quadrats. Soil chemistry of lichen crusts and algal crusts near the study site at Dugway is reported in Table 1. These soils are loams derived from Lake Bonneville sediment. Their chemistry is typical of the fine textured soils of the West Desert in Utah.

Cover of vascular plants and cryptogams prior to disturbance was measured using a quarter meter square nested frequency quadrat. Both weighted frequency and cover according to the point method are reported (Table 2). The readings were actually made within the 108 individual plots. The vascular plant community is dominated by Atriplex confertifolia and Kochia americana, with significant amounts of Sarcobatus vermiculatus as well. Microbiotic crust cover (50%) exceeds vascular plant cover (14%) by a considerable margin. The lichen flora associated with the microbiotic crusts is typical of the light-colored, fine-textured soils of the West Desert. The most common species were Collema tenax, Caloplaca tominii, Catapyrenium lachneum, and Aspicilia species (Table 2). Mosses were present but not identified.

A list of algae observed in the crusts is given in Table 3. The algal flora at Dugway is very diverse, with 79 taxa in the four soils examined. Of these, 21 were Cyanophyta, 37 were Chlorophyta, 6 were Xanthophyta, and 15 were Bacillariophyta. The most frequently observed algae of the area are Microcoleus vaginatus, Nostoc paludosum, Nostoc punctiforme, Tolypothrix byssoidea, Tolypothrix byssoidea var. polycladus, Apatococcus sp. Chlorella vulgaris, Desmococcus vulgaris, Stichococcus dubius, Heterothrix bristoliana, Hantzschia amphioxys, Navicula mutica, Navicula mutica var. cohnii, Navicula mutica var. undulata, and Pinnularia borealis. These algae are all common soil taxa.

Small (1 cm^3) soil samples were removed from the plots to estimate algal abundance within plots. Ten random samples of this size were removed from each quadrat and pooled. The pooled samples were then analyzed for chlorophyll a content using a DMSO extraction method. Although soil samples were collected for chlorophyll a analysis before disturbance of the quadrats, there were problems in the analysis such that we do not feel confident in the data. The method was refined subsequently (Table 4), and samples collected in May 1993 (8 months after trampling/burning) have been analyzed for chlorophyll a. Burning and trampling both had significant effects on algal/lichen abundance as estimated by chlorophyll a. Undisturbed soils had an average of 8.02 ug chl a/g soil, trampled soils had an average of 7.24 ug chl a/g soil, and burned soils had an average of 6.34 ug chl a/g soil. All means were significantly different from one another based on ANOVA and Duncan's Multiple Range Test. Estimation of cyanobacterial biovolumes using epifluorescence microscopy is currently underway.

Sedimentation experiments to determine sedimentation in treated plots was conducted in May 1993. A rain machine was used to apply an even rain to all plots in 2 sedimentation blocks. Rain was applied for 30 minutes to each plot and runoff (liters) as well as sediment removed (grams) was measured. Unfortunately, two of the trampled plots had sampling problems and the data needed to be discarded. Never-the-less, control quadrats showed a significantly lower amount of runoff than disturbed quadrats ($p=.0135$). Sedimentation did not show significant differences, although the burned quadrats appeared to have higher sedimentation than the other two treatments (Table 5). We plan to use more replicates in subsequent sedimentation trials, and expect our method to be better after our initial experience.

Plots were inoculated with cyanobacterial inoculant in May 1993. We used 25 grams/m^2 inoculum consisting of 80% Nostoc and 20% Scytonema. We fear with the absence of rain over the summer that this inoculation will probably not become established. We reinoculated in November 1993, to allow the cyanobacterial amendment to remain on the soil during the colder and wetter winter. For this inoculation, we used 55 grams/m^2, consisting of 45% Nostoc, 45% Microcoleus, and 10% Scytonema. These pellets were ground to a fine powder with a wheat grinder to improve escapability. We will measure establishment and soil stabilization in May 1994.

SUMMARY

This research is progressing excellently. We were able to isolate three cyanobacteria from the site, determine which media were suitable for their growth, and establish appropriate growth conditions. We developed suitable means for growing the algae in mass culture (3 liter flasks). We were able to develop a small-scale methodology for encapsulating the algae in alginate, and produce enough algal pellets for our initial inoculation.

We have thoroughly characterized the study site at Dugway Proving Grounds, and established an objective, systematic, and random sampling strategy for that site. Treatments of trampling and burning have been shown to have significant impacts on the algal biomass (as estimated by chlorophyll a) of the soils, as well as on their sedimentation and runoff properties.

We will finish estimating cyanobacterial biomass using epifluorescence microscopy soon. We plan to examine the algal biomass (chlorophyll a content) and cyanobacterial biomass (epifluorescence microscopy) of the inoculated and uninoculated plots in the summer of 1994.

Table 1. Soil characterization for lichen crust soils and algal crust soils in Dugway Proving Grounds at sites adjacent to the study plots. Soil analyses provided by the Soil Testing Laboratory at Brigham Young University.

Characteristic	Lichen Crust	Algal Crust
Percent Sand	46.28	45.54
Percent Silt	39.91	39.27
Percent Clay	13.80	15.18
Percent Organic Matter	1.49	2.03
pH	7.6	7.8
Electrical Conductivity	0.80	1.94
Sodium Absorption Ratio	1.55	2.44
Nitrate-N (ppm)	5.24	6.25
Phosphate-P (ppm)	13.37	25.23
Exchangeable K (ppm)	328.5	510.9
Soluble Ca (ppm)	72.3	186.0
Soluble Mg (ppm)	26.8	43.0
Soluble Na (ppm)	60.3	133.12

Table 2. Percent cover of vascular plants and other types of cover using the nested frequency method. Cover is estimated using both weighted frequency and the point method.

	Weighted Frequency Percent Cover	Point Method Percent Cover
VASCULAR PLANT COVER		
Atriplex confertifolia	5.72	8.70
Halogeton glomeratus	.30	
Kochia americana	5.32	5.19
Sarcobatus vermiculatus	2.22	1.80
Suaeda torreyana	.57	.37
Unidentified bunch grass	.11	
CRYPTOGAMIC COVER		
Aspicilia species	4.29	
Caloplaca tominii	8.36	.74
Catapyreneum lachneum	4.68	.37
Collema tenax	12.36	12.04
Unidentified moss species	6.36	4.63
Nonlichenized algal crust	13.69	24.26
GENERAL COVER CLASSES		
Shrubs	13.83	16.11
Forbs	.30	
Grasses	.11	
Cryptogamic crust	50.24	42.04
Bare soil	14.94	19.40
Litter	14.87	22.20

Table 3. Algal species observed at Dugway Proving Grounds in a study funded separately from this contract. Presence is indicated with an X.

Species	Uncrusted Sand Dune	Crusted Sand Dune	Lichen Crust	Algal Crust
CYANOPHYTA				
Anabaena variabilis			X	X
Chlorogloea fritschii			X	X
Chroococcus minutus				X
Lyngbya lagerheimii			X	
Microcoleus decolorans			X	
Microcoleus vaginatus	X	X	X	X
Myxosarcina spectabilis				X
Nostoc commune	X	X	X	
Nostoc microscopicum			X	
Nostoc muscorum			X	
Nostoc paludosum	X	X	X	X
Nostoc punctiforme	X	X	X	X
Oscillatoria limosa				X
Oscillatoria quadripunctata			X	
Oscillatoria tenuis	X	X	X	X
Schizothrix calcicola		X	X	
Scytonema millei var. maius	X			
Synechococcus aeruginosus			X	
Tolypothrix byssoidea	X		X	
T. byssoidea var. polycladus	X	X	X	
Tolypothrix campylonemoides			X	
CHLOROPHYTA				
Apatococcus species	X	X	X	X
Bracteacoccus aerius		X	X	
Bracteacoccus aggregata				X
Bracteacoccus giganticus	X		X	
Bracteacoccus grandis			X	
Bracteacoccus minor	X	X	X	
Bracteacoccus species		X	X	
Chlorella mirabilis		X		X
Chlorella protothecoides	X			
Chlorella vulgaris	X	X	X	X
Chlorococcum pulchrum			X	
Chlorosarcinopsis auxotrophica	X			
Chlorosarcinopsis bastropiensis		X		X
Chlorosarcinopsis dissociata				X
Chlorosarcinopsis eremi				X
Chlorosarcinopsis gelatinosa	X			
Desmococcus vulgaris	X	X	X	X
Kentrosphaera grandis		X		
Klebsormidium flaccidum			X	

Table 3. Continued.

Species	Uncrusted Sand Dune	Crusted Sand Dune	Lichen Crust	Algal Crust
Lobococcus incisus	X			
Lobococcus irregularis		X		
Macrochloris species		X		
Myrmecia astigmatica			X	
Palmella texensis	X	X		
Pseudotrebouxia decolorans			X	
Pseudotrebouxia impressa			X	
Pseudotrebouxia species	X			X
Stichococcus bacillaris	X	X	X	
Stichococcus chlorelloides		X	X	
Stichococcus chodatii	X	X		
Stichococcus dubius	X	X		X
Stichococcus palescens	X	X	X	
Tetracystis species 2	X			X
Trebouxia arboricola				X
Trebouxia cladoniae			X	
Trochisiopsis insignis	X			
Trochisiopsis tetraspora		X		
XANTHOPHYTA				
Chloridella polychloris			X	
Heterothrix bristoliana	X	X	X	X
Heterothrix montana			X	
Heterothrix pascheri	X	X		X
Heterothrix sessilis	X		X	X
Heterothrix solida			X	X
BACILLARIOPHYTA				
Aulacoseira granulata	X	X	X	X
Cyclotella species	X	X	X	X
Cymbella mexicana		X		
Denticula elegans	X			X
Diploneis subovalis	X	X	X	
Fragilaria construens var. venter	X			X
Hantzschia amphioxys	X	X	X	X
Navicula contenta var. paralella		X		
Navicula mutica	X	X	X	X
Navicula mutica var. cohnii	X	X	X	X
Navicula mutica var. nivalis		X		
Navicula mutica var. undulata	X	X	X	X
Nitzschia amphibioides			X	
Pinnularia borealis	X	X	X	
Rhopalodia gibba			X	

Table 4. Flow chart for revised method of DMSO extraction of chlorophyll *a*. The centrifuging and filtering are essential steps that must be taken for precise readings.

1. Label plastic centrifuge tubes (15 ml) and glass test tubes (10 ml), with 3 replicates per soil sample.

2. Weigh 1.5 g of soil out for each centrifuge tube.

3. Add 5 ml DMSO to each centrifuge tube of soil, cap and shake thoroughly.

THE FOLLOWING STEPS SHOULD BE PERFORMED IN DIM LIGHT

4. Heat in oven at 65°C for 30 minutes. Remove from oven and shake to dislodge pellet; return to oven for 30 minutes more.

5. Remove from oven and allow to cool in a closed box or other dark area.

6. Centrifuge tubes to completely settle soil pellet.

7. Decant the supernatant into a small filter apparatus with a glass fiber filter and filter the liquid. Pour the filtrate into the appropriate labeled glass tube.

8. Rinse filter apparatus with DMSO, change the filter, and filter next sample. The centrifuge tubes can be set aside to be washed and used again.

9. Read each sample in a spectrophotometer at 665 nm and 730 nm visible light and record absorbances. Each sample should be poured back into its test tube, and the glass cuvettes should be rinsed with DMSO.

10. Acidify each sample with 10 ul of concentrated HCl for 5 minutes and shake to mix.

11. Record absorbance after acidification at 665 nm and 730 nm.

12. If the above steps are followed, and a spectrophotometer cuvette with a beam length of 1 cm is used, then the following formula can be used to determine ug Chl *a*/g soil:

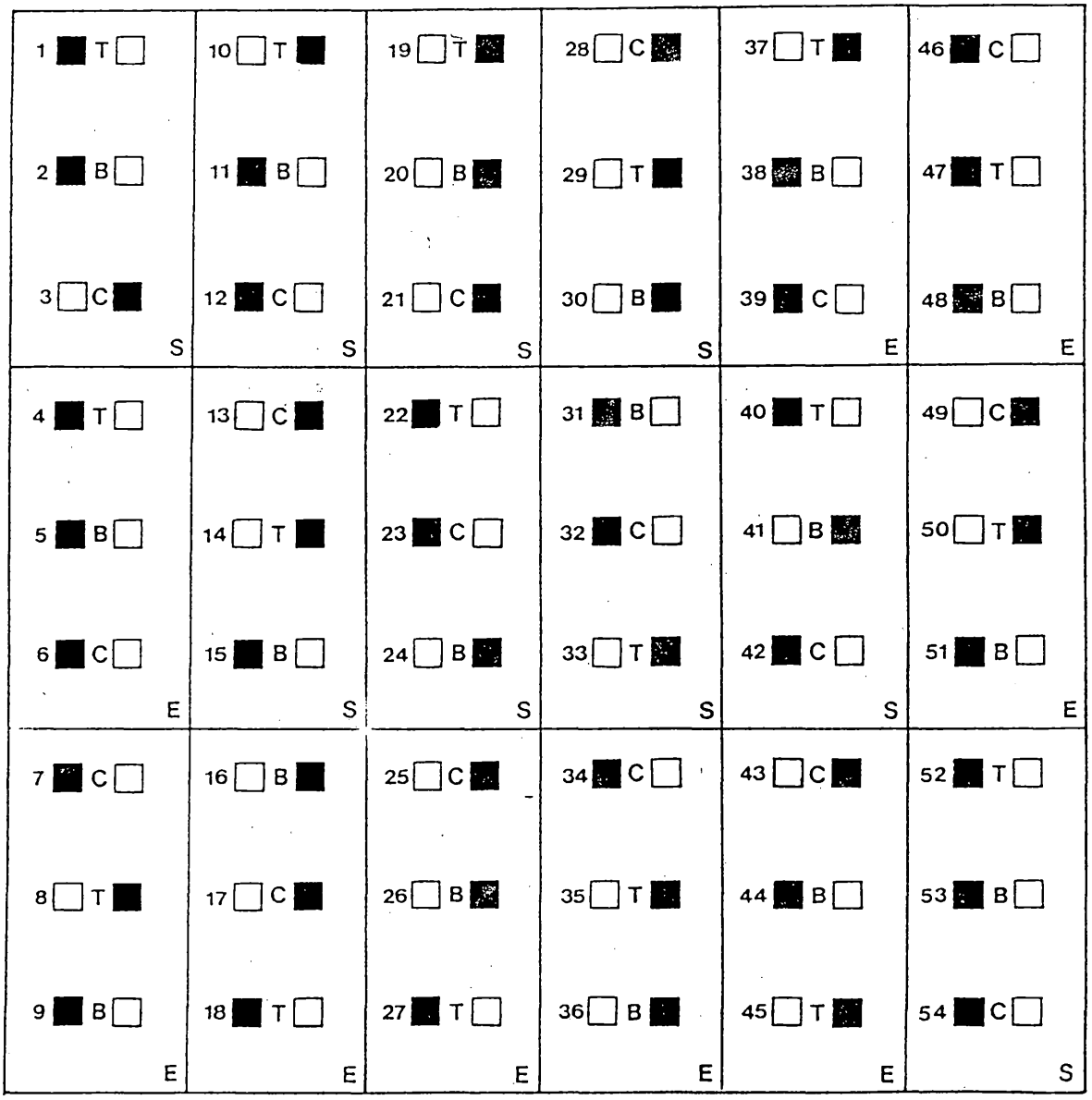
$$\text{Chl } a = 89.3[(665_{before} - 750_{before}) - (665_{after} - 750_{after})]$$

$$\text{Phaeophytin} = 89.3[1.7(665_{after} - 750_{after}) - (665_{before} - 750_{before})]$$

Table 5. Results of initial sedimentation experiments. Water was applied for a period of 30 minutes. The amount of rain varied from trial to trial, and was measured in cm. Two trampled plots had to be discarded. The first received insufficient rain to cause runoff. In the second, water escaped from a poorly sealed catchment device.

Site	Treatment	rain (cm)	runoff (l)	sediment (g)	
19A	trampled	1.6	.012	.616	(discarded)
19B	trampled	2.8	lost	lost	(discarded)
20A	burned	5.4	5.600	14.560	
20B	burned	4.5	10.260	73.103	
21A	control	3.2	4.600	6.919	
21B	control	3.2	2.900	3.757	
25A	control	3.5	9.750	24.119	
25B	control	3.8	0.017	.097	
26A	burned	2.8	5.870	32.482	
26B	burned	4.9	9.870	31.146	
27A	trampled	6.3	11.270	9.773	
27B	trampled	6.6	10.710	6.887	
Means					
	trampled	6.5	10.99	8.33	
	burned	4.4	7.90	37.82	
	control	3.4	4.32	8.72	

Figure 1. Map of plots showing treatment assignments. There were 54 pairs of plots in 18 blocks. Two treatments are shown. The disturbance treatment was applied to pairs of plots and had 3 levels (T=trampled, B=burned, C=control). The inoculation treatment had two levels (Inoculated = black squares, Not inoculated = hollow squares) which were assigned to every pair of plots. Half the 18 blocks were designated as sedimentation sites (S in lower right corner), with the other half set aside for algal enumeration (E in lower right corner). All treatments were assigned randomly before setting up the study grid at Dugway Proving Grounds.



DEVELOPMENT OF TECHNOLOGY AND EQUIPMENT FOR MASS PRODUCTION OF CYANOBACTERIAL PELLETS AND GREENHOUSE TESTING OF PELLET VIABILITY AND BEST POSSIBLE APPLICATION PROCEDURES REPORT

Prepared by

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INTRODUCTION

In our first project (Propagation of Cryptogams for Use in Land Stabilization) we developed the methods to grow selected cyanobacteria in liquid culture and encapsulate these algae in alginate pellets. However, the methods developed were labor-intensive and produced only small quantities of pelletized cyanobacteria. We perceived a need to be able to grow the cyanobacteria in mass quantities with less labor/yield and less cost/yield. This report presents the results of our efforts.

RESEARCH OBJECTIVES

1. Scale up production of cyanobacteria pellets.
2. Conduct greenhouse/growth chamber studies to evaluate viability and longevity of cyanobacteria pellets.
3. Conduct greenhouse/growth chamber studies to evaluate inoculation and escape success of pelletized cyanobacteria.
4. Conduct greenhouse/growth chamber studies to determine minimum moisture levels for crust establishment.
5. Conduct greenhouse/growth chamber studies to determine soil fertility enhancement following application of cyanobacteria pellets to study plots.
6. Conduct greenhouse/growth chamber studies to determine best possible application procedures for pelletized cyanobacteria.

MASS PRODUCTION OF CYANOBACTERIA

Previously we grew cyanobacteria in small flasks, which were used to inoculate 3 L Bellco stirrer flasks. We purchased four 250 liter flat-bottom, cylindrical, plexiglass tanks from the Solar Components Corporation, 121 Valley St., Manchester, NH 03103. These tanks were 5 feet high and 18" in diameter and came with friction fit lids. The major problems anticipated in upscaling our production using the larger tanks were: 1) production of sufficient quantities of Z-8 media, 2) contamination by algae-bearing dust particles from the air, 3) adequate aeration of such large quantities of media, and 4) providing an adequate light source. A description of how we dealt with these potential problems follows.

We developed a method of mass production of Z-8 media, which requires filter sterilization. First, we autoclave a 4 liter filter apparatus, a pack of Gelman 0.45 um Metricel membrane

filters, and a 20 liter carboy. Flame sterilized tweezers are used to insert a filter into the filter apparatus. Second, 16 liters of distilled water are filtered. The 16 liters of sterile water is transferred to the sterile carboy four-liters at a time. The lip of the flask is flamed sterilized each time before the water is transferred. Third, we began filtering the mineral stock solutions. The trace stock (20 ml) is sent through first, followed by distilled water to rinse. Then each of the first four macronutrient stocks (200 ml each of : Fe-EDTA, $NaNO_3$, K_2HPO_4, Na_2CO_3) is sent through, again rinsing with distilled water after each nutrient solution. The total amount of distilled water used for the rinses in this step is 1800 ml. At this point the filter is replaced using sterile technique. Fourth, we filter another 1000 ml. of distilled water. We filter the last two stock solutions (200 ml each of: $Ca(NO_3)_2$, $MgSO_4$) into the 1000 ml of distilled water, rinsing after each solution with the remaining 1000 ml of distilled water thus bringing the total volume in the carboy to 20 liters. Finally, the carboy is shaken to thoroughly mix the solution.

Later we tested cyanobacterial growth in media prepared without distilled water. Dechlorinated tap water (treated with aquarium dechlorinator) was used instead of distilled water. We did not filter-sterilize the media made from tap water. Growth in this media was excellent, and no contamination occurred. We only used nonsterile media once we had a substantial (more than 40 liters) culture growing in the 250 L tanks. The cyanobacteria in a thick culture cause the pH to rise, preventing the invasion of green coccoid contaminants.

Although we had no contaminants in our mass culture tanks, it is still a potential problem. The precautions we took which may be a part of our success were: 1) cultures were started in sterile media, and replenished with sterile media until at least 40 liters were in the tank, 2) the air which aerated the tank was passed through a sterile Gelman ACRO 50 disposable PTFE filter (0.45 um), 3) the friction-fit lid had an air outlet that vented horizontally, so that dust did not fall into the culture through the outlet hole, 5) between culturing cyanobacteria, the tank was cleansed with a Chlorox solution to kill any residual cyanobacteria left in the tank (since the tanks are not autoclavable).

Aeration was accomplished by purchasing an automobile pollution control pump and hooking this up to a ⅓ HP electric fan motor. PVC pipe (¾ in) was used to pipe the air from the pump into the tank. The aeration pipe was constructed such that it fed into an H-shaped PVC piece that rested on the bottom of the tank. Small holes (1/32 inch) were drilled in the H-shaped piece to allow air to escape. Aeration was excellent, with considerable lift, so that cultures were both adequately aerated and agitated by the device. The ACRO 50 air filter was replaced when aeration was noted to slow down due to clogging of the filter.

Light was supplied by two vertically-mounted four-foot fluorescent shop lights per tank. The lights were set 5 inches from the outside surface of the tank. Continuous lighting was used.

We have cultured 2 of the three cyanobacterial taxa in the large tanks at John Carroll University. First, we cultured Nostoc punctiforme. One tank was inoculated with 12 liters of culture from the Bellco stirrer flasks. At the time of inoculation, 20 liters of fresh media were added. Additional media was added every 2-3 days, with the amount of media added increasing over time. We filled the 250 liter tank with about 220 liters, then split the tank so

that the two tanks had 110 liters. We then took these up to 220 liters each. This whole process took about three weeks. Once the tanks were full, 20-40 liters could be removed each day and harvested, with the addition of fresh media making up for the loss. This could be sustained for a longer period of time than we deemed necessary. The main problem at this point is the harvesting of the algae, which is much more labor intensive than the maintenance of the algal culture.

The second species we grew was Microcoleus vaginatus. This taxon grew as rapidly as the Nostoc in the big tanks, even though we had some problems growing the algae in smaller cultures. High aeration and lower light intensities seem to flavor its growth. We had no problems with contamination by either green coccoid algae or other cyanobacteria (including Nostoc, which had been grown in the tanks previously).

HARVESTING AND PELLETIZING CYANOBACTERIA

In order to pelletize the algae more rapidly, a plexiglass drip-tank was constructed. This tank holds 80 liters of liquid, and is pierced on the bottom by over one-hundred holes, which are fitted with plastic pipette-tips so that the alginate drips through in similar sized drops. A tank of the same capacity is placed below the drip tank. This tank holds the calcium chloride solution which causes the alginate drops to precipitate into pellets. It is fitted with a drain so that the liquid can be removed when needed. A screen over the drain opening allows water to leave without loss of the pellets.

At the beginning of the process, two liters of the cyanobacterial culture were removed from the tank and filtered through a No. 230 copper sieve. The algae was weighed so that the culture could be diluted properly (2.5 g/liter). A total of 20 or 30 liters of diluted culture was mixed with alginate to make a 1% solution (10 g Kelgin/liter). This was done with a kitchen blender to assure effective mixing of the alginate. The bottom tank was filled with a 2% $CaCl_2$ solution, in the same quantity as the alginate solution to be pelletized (20 or 30 liters). At first we used reagent grade calcium chloride, but later found that Ice-Melt, a low grade calcium chloride for melting ice on sidewalks, works as well as reagent grade calcium chloride for a fraction of the cost. When the solution was in place in the lower tank, the upper tank was filled with the alginate solution. The 20-30 liters of the diluted culture dripped through in less than an hour.

The pellets must remain in the calcium chloride solution for at least an hour to set up properly. The liquid was then drained off and the tank filled with unsterile Z-8 media to assist in removing excess $CaCl_2$. The pellets were soaked in the media for at least 3 hours. The media was then drained off and the pellets were ready to be dried.

We constructed a rack to hold 60 cafeteria trays for drying pellets. Pellets were spread evenly on the trays. Each day we came in and broke-up the pellets so they would not form large aggregates. Drying took 1-3 days, depending on temperature of the room, humidity, and air circulation. The pellets were then placed in air-tight bottles and refrigerated.

Currently, we are limited in production of pellets by the drying stage. We could use 3 additional drying racks, and possibly another drip tank to be able to keep up with algal production in the big tanks. These items were relatively inexpensive, and so we may go ahead and construct these items if we have need of making sufficient pellets to stabilize large areas of land. If we do indeed decide this is the best methodology we have established that we can produce unlimited quantities for a reasonable cost. At this time, we have not priced what the pellets cost per kg in terms of labor and supplies. This information could be gathered now that the methods have been worked out.

GREENHOUSE/GROWTH CHAMBER STUDIES

In order to evaluate viability and longevity of pelletized cyanobacteria 15-20 pellets were periodically placed on wet filter paper in sterile plastic petri dishes. Samples were kept under fluorescent light at 21° C for one week. At three days, five days and 7 days the pelletized cyanobacteria were evaluated using an Olympus SZH dissecting microscope. The two species of pelletized cyanobacteria (Scytonema hofmannii and Nostoc punctiforme) used in this experiment remained viable throughout the study; however, it became increasingly apparent that once the alginate pellets dried out they would not completely rehydrate. Careful examination of this problem showed that calcium ions, from the calcium chloride solution used to precipitate the alginate, replaced the sodium ions in the alginate matrix thus inducing pellet formation. We discovered that in order to reverse the process so that the pellets would fully rehydrate they had to be soaked in a sodium chloride solution. This requirement obviously draws into question the advisability of using alginate as an inoculation vector. Growth chamber studies, where cyanobacteria pellets were applied to the surface of a greenhouse soil (mixed to simulate the chemistry and texture of the soils at the Dugway site), showed the same problems with pellet rehydration. Again the cyanobacteria in the pellets remained viable but were not able to escape the hardened alginate matrix. These complications resulted in a preliminary set of laboratory studies comparing various alternative methods for dispersing the cyanobacteria. Four different methods were evaluated: 1) freeze-dried, alginate pellets; 2) powdered, air-dried alginate pellets; 3) air dried, powdered cyanobacteria; and 4) cyanobacteria dried onto the soil mix used in the previous greenhouse studies. Standard alginate pellets were used as a control. Criteria for evaluating the various methods centered on whether or not the cyanobacteria were able to develop independent of the dispersal vector. Freeze-dried, alginate pellets rehydrated significantly better than the air-dried pellets; however, the cyanobacteria were still unable to escape from the alginate matrix. Cracking/powdering the air-dried alginate pellets, using a wheat grinder, significantly increased the escape capacity of the cyanobacteria (apparently cracking the alginate pellets creates fractured surfaces with exposed cyanobacteria filaments, which are then able to move out of the alginate matrix). Air-dried powdered cyanobacteria rehydrated nicely and quickly produced actively developing algal filaments; but without a carrier medium there are significant dispersal complications. The greenhouse soil served as an effective dispersal vector. The cyanobacteria dried onto the surface of the soil rehydrated effectively and quickly produced actively growing colonies. Followup studies are necessary to accurately determine which of these methods will yield the best and most cost effective technical results.

SUMMARY

We have successfully developed the technology and equipment to mass produce cyanobacterial pellets. We use 250 liter cylindrical tanks, aerated with PVC pipe connected to a pollution control air pump run by a fan motor. A drip tank with an accompanying calcium chloride tank and stand have been set up. Pellets are dried on trays placed in a drying rack. We have proven that this system works with two of the genera of cyanobacteria. Pellets have been mass produced for field studies. This past November powdered, alginate pellets of all three cyanobacterial species were used to inoculate the field plots at Dugway Proving Grounds. We expect to gather field data to evaluate the effectiveness of the powdered pellets during the summer of 1994 under funding from a separate, related project.

EFFECTS OF CRYPTOBIOTIC SOIL CRUSTS AND VA MYCORRHIZAL FUNGI ON GROWTH OF FIVE RANGELAND PLANT SPECIES

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Cryptobiotic crusts of arid and semiarid lands contribute significantly to ecosystem stability by means of soil stabilization and improved growth and establishment of vascular plant species (Harper and Marble 1988; St. Clair and Johansen 1993). Their importance as a source of biologically available nitrogen in arid systems is well-established; fixation rates are estimated at 10-100kg $Nha^{-1}yr^{-1}$ (Rychert et al. 1978). Harper and Pendleton (1993) present possible mechanisms for enhanced seedling establishment and growth, including increased nutrient content of soil surface layers, higher soil temperatures during early-season growth, and improved availability of essential elements due to chelating compounds present in cyanobacterial sheaths. They also found that plants growing on crusted soils had significantly higher levels of colonization by vesicular-arbuscular (VA) mycorrhizal fungi and rhizosheath-producing microorganisms. VA mycorrhizae are known to aid plants in nutrient acquisition, primarily of phosphorus, but their role in desert systems is poorly understood. Colonization, although frequently present, may vary widely with season (Allen 1983), possibly corresponding to changes in photosynthetic activity (Bethlenfalvay et al. 1984). This study was initiated for the purpose of examining the combined effects of mycorrhizal fungi and cryptobiotic crust organisms on the growth and nutrient content of arid-land plant species.

MATERIALS AND METHODS

Five plant species, including an annual grass (Bromus tectorum), a perennial grass (Sitanion hystrix), an annual forb (Gaillardia pulchella), a perennial forb (Sphaeralcea munroana), and a shrub (Coleogyne ramosissima) were used in the experiment. Seed sources are given in Table 1. Seeds of the first four species listed above (BRTE, SIHY, GAPU, and SPMU) were sown 3-4 per cell in 6-celled books (Ferdinands from Hummert) filled with a steam-sterilized sandy loam from Grand County, Utah. Cells were later thinned to one plant per cell. At two weeks, plants were transplanted to 6-inch pots containing one of six soil/inoculum treatments. Seeds of Coleogyne ramosissima (CORA) were imbibed on blue blotter paper in petri dishes and cold stratified at 4C for two weeks. Germlings were transplanted directly into soil treatments.

Three soil treatments were used in the experiment: blow sand, mixed crust, and crust material placed over the top of blow sand. Blow sand collected from between plants at Sand Flats near Moab, Utah, was steam-sterilized at 65C for one hour. Crust material for the mixed crust treatment was collected near sandstone outcrops at the same location and thoroughly mixed using a small cement mixer. Six-inch circles of crust were collected in large petri dishes from Behind-the-rocks, also near Moab, and placed over the top of the steamed sand to make up the third soil treatment. The following lichens were identified from the crust circles by Dr. Larry St. Clair of Brigham Young University: Collema tenax, Fulgensia fulgens, Catapyrenium lachneum, Psora decipiens, Squamarina lentigera, and Buellia elegans. Moss of the genus

Table 1. Seed sources for plant species used in the greenhouse experiment.

Species	Source location
Bromus tectorum	Shrub Lab farm, east bench, Provo, Utah
Sitanion hystrix	Browse exit, I-15, Washington County, Utah
Gaillardia pulchella	Dick Page, BLM; obtained from Granite Seed Co.
Sphaeralcea munroana	I-70, 41 miles east of Green River, Utah
Coleogyne ramosissima	Washington County, Utah

Table 2. Soil characteristics for components of the three soil treatments. Mixed bulk samples were used for blow sand and mixed crust treatments. For the crust over sand treatment, the mean and range representing five crust circles are given.

Characteristic	Blow sand	Mixed crust	Crust circles
Reaction (pH)	7.4	7.2	7.2 (7.0-7.5)
Organic matter (%)	0.45	1.23	1.88 (1.37-2.17)
Sodium absorption ratio	0.03	0.08	0.67 (0.46-1.15)
Conductivity (Ec x 10^3)	0.72	1.48	1.81 (1.26-2.25)
- - - - - - - - - - PPM "available" - - - - - - - - - - - - -			
Calcium	184.5	231.0	249.1 (156.5-403.0)
Copper	0.18	0.24	0.45 (0.34-0.58)
Iron	17.10	3.56	3.63 (3.08-4.64)
Magnesium	15.0	41.5	63.0 (40.5-94.5)
Manganese	9.38	10.98	7.24 (6.28-8.46)
Nitrate-nitrogen	4.06	79.54	34.62 (8.16-104.23)
Phosphorus	12.18	7.53	9.81 (6.12-12.45)
Potassium	147.2	108.8	160.64 (102.4-201.6)
Sodium	1.60	5.12	43.62 (26.56-63.52)
Zinc	2.16	0.22	0.47 (0.40-0.54)

Tortula and various algae, including Microcoleus vaginatus were also present. Soil properties of the treatment components are given in Table 2.

Mycorrhizal inoculum of the species Glomus intraradices was added to half of the pots in each soil treatment, making a total of six soil/inoculum treatment combinations. Ten plants of each species were used in each treatment combination for a total of 300 plants. Inoculum was obtained from Brokaw Nursery in Saticoy, CA., Approximately 1 1/2 tablespoons of the inoculum (300-700 spores) was added to the root zone of treated plants at the time of transplanting.

Plants were maintained in a glasshouse during the summer months of 1993. Pots were bottom watered as needed using a capillary matting system fitted with drip irrigation (Hummert International). Plants were sprayed three times during the experiment with Cygon 2E systemic (2 teaspoons per gallon) to prevent aphid infestation. Plants were harvested at the end of 10 weeks, with the exception of CORA, which was grown for 12 weeks. Shoots were excised at ground level, dried at 65C, weighed, and the leaves ground for nutrient analysis. Reproductive tissue, when present, was weighed separately. Leaf tissue analysis was done by the Soil and Plant Testing Laboratory, Brigham Young University. Heights of GAPU and SPMU plants were also recorded. Roots were washed and fixed in 70% denatured ethanol. Subsequently, roots were picked free of sticks and other debris, dried at 65C, weighed, and rehydrated in fixative for pending analysis of mycorrhizal colonization and root architecture.

Root and shoot vegetative biomass, reproductive biomass, total plant biomass, plant height, and root/shoot ratios were analysed for each species using the ANOVA procedure from SAS for personal computers (SAS Institute, Inc. 1988). The model used was a 3 x 2 factorial, with soil treatment and VA mycorrhizal inoculum as the main effects. Mean separations were accomplished using the Student-Newman-Keuls multiple range test. Where the soil*VAM interaction was significant, mycorrhizal and nonmycorrhizal plant measures were compared separately for each soil level.

RESULTS

Results from the ANOVAs are given in Table 3. Soil treatment had a significant effect on nearly all variables examined. Mycorrhizal inoculum had a significant effect on less than half of the variables examined and was highly species dependent. The interaction term was significant in only two cases, both root variables of Gaillardia pulchella. The results for each species will be presented separately below.

Bromus tectorum was highly responsive to crust additions to the soil. The mixed crust soil treatment produced the greatest amount of root and shoot biomass (Table 4), but plants from the crust over sand treatment also had significantly greater biomass than plants grown in blow sand. This most likely represents a response to the increased nitrogen levels of soils with crust additions. Percent organic matter, magnesium, and nitrate-nitrogen were all substantially improved in crusted soils (Table 2). Bromus tectorum responds favorably to high levels of nitrogen, remaining dominant on disturbed sites where nitrogen is readily available (McLendon and Redente 1991). Of particular interest in this regard was the enormous quantity of fine feeder roots which

Table 3. Attained significance values from ANOVAS for six growth parameters of five plant species. Soil type and mycorrhizal inoculum (VAM) were used as main effects.

Species/effects	Vegetative shoot wt.	Reproductive shoot wt.	Total shoot wt.	Root wt.	Total plant wt.	Root/shoot ratio	Height
BRTE							
soil	0.0001	---	---	0.0001	0.0001	0.0001	---
vam	NS	---	---	0.0005	0.0124	0.0001	---
soil*vam	NS	---	---	NS	NS	NS	---
SIHY							
soil	0.0001	0.0004	0.0001	0.0797	0.0001	0.0001	---
vam	0.0233	NS	NS	0.0001	0.0171	0.0001	---
soil*vam	NS	NS	NS	NS	NS	NS	---
GAPU							
soil	0.0001	0.0510	0.0001	0.0001	0.0001	0.0006	NS
vam	NS	NS	NS	0.0241	NS	NS	NS
soil*vam	NS	NS	NS	0.0091	NS	0.0027	NS
SPMU							
soil	0.0001	0.0001	0.0001	---	---	---	0.0001
vam	NS	NS	NS	---	---	---	NS
soil*vam	NS	NS	NS	---	---	---	NS
CORA							
soil	0.0420	---	---	0.0067	0.1857	0.0001	---
vam	0.0100	---	---	NS	0.0208	0.0496	---
soil*vam	NS	---	---	NS	NS	NS	---

Table 4. Mean values for plant growth measurements from three soil treatments. Letters following means indicate significant differences (p = 0.05) among values. Biomass values are given in grams, height in cm.

Species/effects	Vegetative shoot wt.	Reproductive shoot wt.	Total shoot wt.	Root wt.	Total plant wt.	Root/shoot ratio	Height
BRTE							
mixed crust	3.87 a	---	---	1.33 a	5.20 a	0.31 a	---
sand with crust	1.08 b	---	---	0.66 b	1.74 b	0.60 b	---
blow sand	0.51 c	---	---	0.39 c	0.91 c	0.74 c	---
SIHY							
mixed crust	1.25 a	0.45 a	1.69 a	0.44 a	2.14 a	0.26 a	---
sand with crust	0.46 b	0.002 b	0.47 b	0.37 a	0.83 b	0.68 b	---
blow sand	0.30 c	0.001 b	0.30 c	0.23 a	0.53 c	0.68 b	---
GAPU							
mixed crust	4.41 a	0.10 a	4.51 a	1.36 a	5.87 a	0.34 a	28.9 a
sand with crust	0.78 b	0.03 a	0.81 b	0.35 b	1.16 b	0.46 b	19.7 a
blow sand	0.73 b	0.02 a	0.75 b	0.32 b	1.07 b	0.46 b	19.1 a
SPMU							
mixed crust	3.53 a	0.26 a	3.79 a	---	---	---	32.0 a
sand with crust	0.42 b	0.0 b	0.42 b	---	---	---	3.6 b
blow sand	0.31 c	0.0 b	0.31 c	---	---	---	2.7 c
CORA							
mixed crust	0.21 a	---	---	0.04 a	0.25 a	0.19 a	---
sand with crust	0.19 ab	---	---	0.06 b	0.25 a	0.34 b	---
blow sand	0.15 b	---	---	0.05 b	0.20 a	0.40 c	---

Table 5. Mean values for plant growth measurements from mycorrhizal and nonmycorrhizal plants. Letters following means indicate significant differences (p = 0.05) between values. Biomass values are given in grams, height in centimeters.

Species/effects	Vegetative shoot wt.	Reproductive shoot wt.	Total shoot wt.	Root wt.	Total plant wt.	Root/shoot ratio	Height
BRTE							
+ VAM	1.46 a	---	---	0.50 a	1.96 a	0.46 a	---
- VAM	2.18 a	---	---	1.09 b	3.27 b	0.65 b	---
SIHY							
+ VAM	0.50 a	0.14 a	0.65 a	0.15 a	0.80 a	0.36 a	---
- VAM	0.84 b	0.16 a	0.99 a	0.54 b	1.53 b	0.72 b	---
GAPU							
+ VAM	1.99 a	0.04 a	2.03 a	0.60 a*	2.64 a	0.41 a*	21.8 a
- VAM	1.95 a	0.06 a	2.01 a	0.75 b	2.76 a	0.43 a	23.8 a
SPMU							
+ VAM	1.48 a	0.10 a	1.58 a	---	---	---	13.5 a
- VAM	1.37 a	0.08 a	1.44 a	---	---	---	12.0 a
CORA							
+ VAM	0.21 b	---	---	0.06 a	0.26 b	0.29 a	---
- VAM	0.15 a	---	---	0.05 a	0.20 a	0.33 b	---

* Significant interaction term. See text for an explanation.

grew up into the crust itself in the crust over sand treatment, comprising 43% of the total root biomass for nonmycorrhizal plants. None of the other species mined the crust for nutrients in this fashion. The root/shoot ratio also decreased with increasing crust additions, indicating that the plant invested proportionately fewer resources as nutrient availability increased.

Inoculation with mycorrhizal fungi produced a negative growth response in Bromus at all three soil levels (Table 5). Root and shoot biomass both declined in the presence of VAM, however, the roots were reduced proportionately more. As a result, the root/shoot ratio of mycorrhizal plants was significantly lower than that of nonmycorrhizal plants (Fig. 1d). Also, mycorrhizal plants did not mine the crust of the crust over sand treatment as did nonmycorrhizal plants. Mycorrhizal plants had a mean of only 0.04 g of root biomass harvested from the crust itself as opposed to 0.40 g of root biomass for nonmycorrhizal plants ($p = 0.0005$).

Soil treatment also had a pronounced effect on Sitanion hystrix (Table 3) for all measures of above-ground biomass. The mixed crust treatment produced the most vegetative shoot biomass, reproductive biomass, total shoot biomass, and total plant biomass (Table 4). Mixed crust plants not only grew faster, but also began flowering much sooner than plants of the other two treatments. Very little reproductive tissue had been produced in either of the other soil treatments at the time of harvest. Because of the over 200 percent growth increase of the mixed crust treatment over that of the blow sand (Fig. 1a), the root/shoot ratio for this treatment was much lower than that of other treatments. Although root biomass of the mixed crust treatment tended to be higher than that of the other treatments, it was not significantly different. The crust over sand treatment produced significantly more vegetative shoot biomass, total shoot biomass, and total plant biomass than did the blow sand treatment. Root biomass and root/shoot ratios did not differ between these two treatments.

Mycorrhizal inoculum again produced a negative growth response in Sitanion. Vegetative shoot, root, and total plant biomass were all significantly lower in inoculated plants (Table 5). Indeed, while plants were relatively small, sustaining the fungi apparently drained plant resources to the point of death. Ten of thirty Sitanion plants inoculated mycorrhizal fungi died in the first three weeks of the experiment and had to be replanted. One of the replacements subsequently died and was not replaced. This mortality was not related to soil treatment and occurred only in Sitanion. Only two other plants (one BRTE and one CORA) died during the experiment. Reproductive biomass was not affected by inoculation. The mean root/shoot ratio for mycorrhizal plants was again significantly lower (approximately half) than that of nonmycorrhizal plants.

For Gaillardia pulchella, the mixed crust treatment produced significantly larger measures of vegetative shoot biomass, total shoot biomass, root biomass, and total plant biomass (Table 4). The root/shoot ratio was significantly lower. Reproductive biomass for the mixed crust treatment was larger, but not statistically significant because of high variability among plants. Unlike SIHY, some reproduction occurred at all soil levels. Plant height, which is related to flowering in this species, also did not vary significantly, although larger in the mixed crust treatment. No significant treatment effects were observed between the blow sand and crust over sand treatment levels.

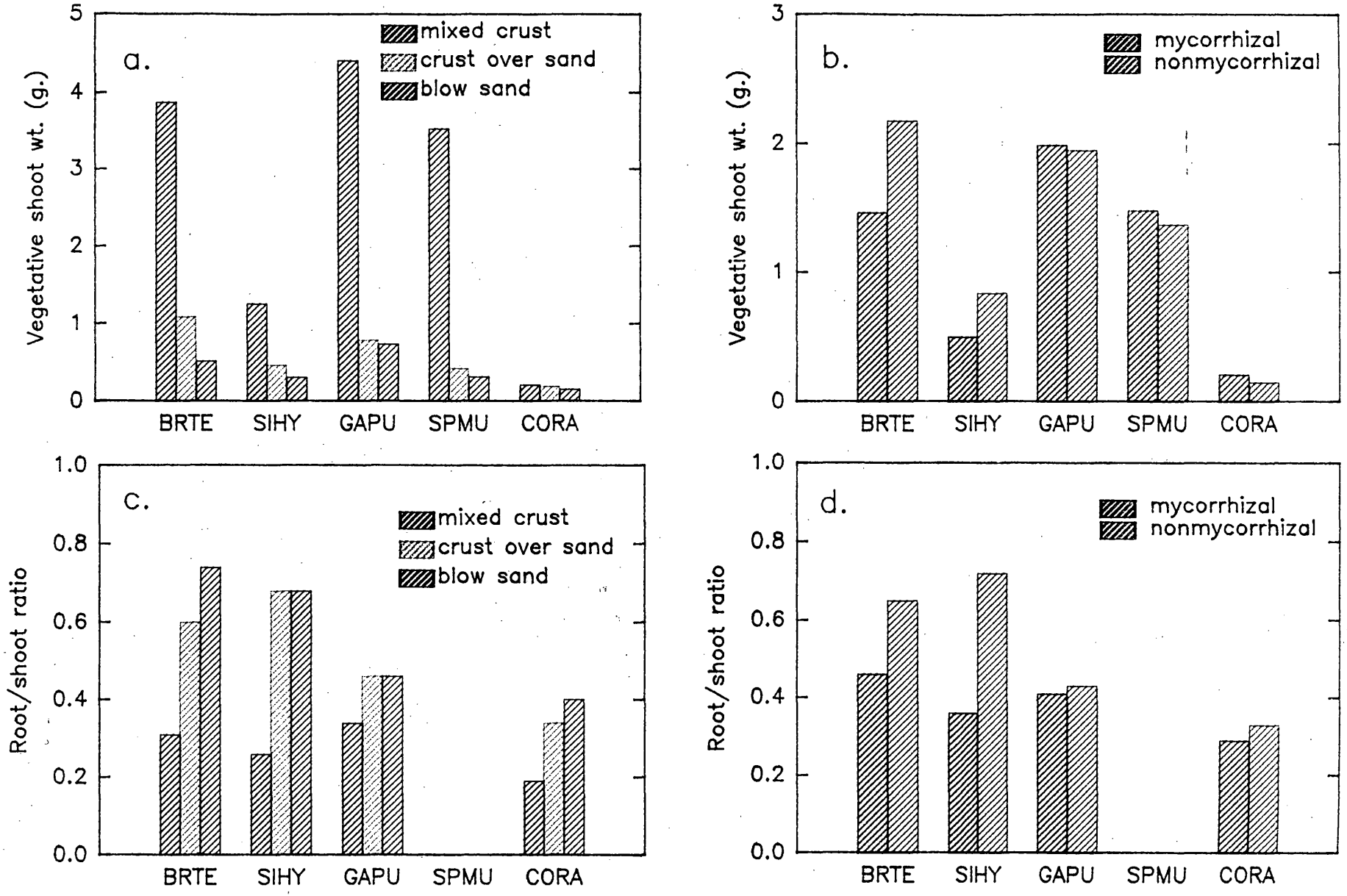

Fig. 1. Soil and VAM inoculation treatment means for vegetative shoot weight and root/shoot ratio variables of five plant species.

At first glance, it appears that mycorrhizal inoculum produced no effect on growth of Gaillardia plants (Table 5). However, the interaction terms for the two root variables (root biomass and root/shoot ratio) were significant (Table 1). When mycorrhizal and nonmycorrhizal plants were compared at each soil level, it was found that significant differences occurred only in the mixed crust soil treatment. At this level, the mycorrhizal plants produced less root biomass (1.14 g compared with 1.58 g; $p = 0.0285$) and had lower root/shoot ratios (0.26 compared with 0.41; $p = 0.0024$). There was also a consistent trend for mycorrhizal plants to have higher biomass values in the two crust treatments and lower values in blow sand than did nonmycorrhizal plants. For example, mean values for vegetative shoot weight of mycorrhizal and nonmycorrhizal plants were 4.58 and 4.25 in the mixed crust treatment, 0.81 and 0.74 in the crust over sand treatment, and 0.59 and 0.86 in the blow sand treatment. This intriguing trend, suggestive as it is of further crust/VAM interaction, may be worth more intensive study.

The root data for Sphaeralcea munroana are not yet complete. Root biomass, total plant biomass and root/shoot ratio figures were not available for analysis, except for the blow sand treatment. Shoot growth variables, including vegetative shoot weight, reproductive shoot weight, total shoot weight, and shoot height were significantly larger for the mixed crust treatment (Table 4). Although more modest, significant growth differences also occurred between crust over sand and blow sand treatments. No reproduction occurred at either of these two soil levels.

Inoculation with mycorrhizal fungi had no significant effect on Sphaeralcea plants (Table 1). There was a slight but consistent trend for mycorrhizal plants to be larger in the crust over sand treatment, but not in the mixed crust or blow sand treatments. Root data from the blow sand treatment showed no significant differences between mycorrhizal and nonmycorrhizal plants for either root biomass or root/shoot ratio variables.

Due to its slower rate of growth, soil treatment had a significant but less dramatic effect on Coleogyne ramosissima when compared with other plant species used in the study. Although total plant weight did not differ among soil treatments (Table 4), plants grown in the mixed crust treatment had larger shoots (although significantly different only from those grown in blow sand) but smaller roots than those from other soil treatments. No significant differences occurred between crust over sand and blow sand treatments for these three variables. Root/shoot ratios differed significantly among all three soil treatments, with mixed crust having the smallest mean and blow sand having the greatest.

Coleogyne plants showed a positive growth response to mycorrhizal inoculum. Both shoot weight and total plant weight were significantly greater for mycorrhizal plants (Table 5). Root weight did not differ between treatments. The root/shoot ratio was also significantly lower in mycorrhizal plants. Coleogyne was the only species in this study to show a definite positive response to inoculation.

DISCUSSION

These results suggest a certain similarity within life form in their response to study treatments. The grasses responded to both levels of crust addition by

increasing shoot and root growth (Fig. 1). Both grasses responded negatively, however, to inoculation by mycorrhizal fungi, decreasing both root and shoot mass. The forbs increased growth slightly in response to the circle of crust added over sand. Growth was increased dramatically in the mixed crust treatment. In contrast with the grasses, the forbs showed a slight but nonsignificant growth response in the presence of mycorrhizae. The shrub, Coleogyne, showed a much less dramatic response to crust additions (Fig. 1). Like other species, top growth increased in the presence of crust, but unlike other species, root growth decreased (Table 4). Coleogyne showed the only significant positive growth response to mycorrhizal inoculation.

All species used in this study showed a decline in root/shoot ratios at the highest soil nutrient levels (Fig. 1b). Half of the species for which data are available (BRTE and CORA) also demonstrated a decline in root/shoot ratios when grown in the presence of a crust layer. These data support other studies, which have found that the relative proportion of shoot weight to total plant weight increases as soil fertility goes up (Chapin 1980). This is interpreted to mean that, at higher nutrient availability, proportionately less of the root system is needed to meet the demand for plant growth (see Kachi and Rorison 1989 for a discussion of this topic).

All species showed a similar decline in root/shoot ratio in the presence of mycorrhizal fungi. This decline occurred regardless of whether or not inoculation produced a positive growth response. Mycorrhizal fungi essentially provide an extension of the root surface area, presumably at lower cost to the plant. Plants which form mycorrhizal associations might, therefore, be expected to invest proportionately fewer plant resources in the production of root tissue than their nonmycorrhizal counterparts. However, study results have been mixed, with reports of both increasing and decreasing root/shoot ratios (Allen 1991). Differing results may be obtained depending on the plant species used and the chemical environment in which the plant is grown (Allen 1991). Recent studies report that colonization by VAM fungi cause changes in root architecture (Hetrick et al. 1991; Hooker et al. 1992), an approach we intend to use in subsequent data collection.

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